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E. coli control in manufacturing beef – US study tour by a processor study group

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Abstract

Following the declaration of six additional E. coli serogroups (STECs) as adulterants, Australian processors expressed a need to increase their understanding of the options available to respond to these requirements and respond to presumtive positive and confirmed positive results for STECs. This project involved a study program for meat processing quality assurance and operations managers in the USA, designed to examine interventions applied on plant, participate in North American Meat Association (NAMA) conference and hold specific meetings with supply chains using grinding beef. The group prepared this report compiling the results of these meetings under various topical headings, drawing some conclusions about areas that Australian processors need to consider, and making suggestions for further research.

Executive Summary

Australian meat is regarded internationally as having a very high standard of safety and the systems used to raise animals, process and transport them are seen by trading partners as being outcomes-based, innovative and effective. Recently, the United States FSIS established new rules relating to certain Shiga toxin-producting Escherichia coli (STECs), which requires the application of process control, HACCP, and testing to manage these risks. Processors in the US are applying a range of different interventions to manage risks as well as a range of quality assurance (including testing) responses to address these new requirements. In Australia, there is a need to increase the understanding of the options available to respond to these requirements and respond to presumtive positive and confirmed positive results for STECs.

The project involved a study program for meat processing quality assurance and operations managers in the USA, designed to examine interventions applied on plant, participate in North American Meat Association (NAMA) conference and hold specific meetings with supply chains using grinding beef.

At the end of the tour the group reviewed the developing report and selected and collated the following important messages for the broad Australian processing sector:

- There is unlikely to be any change in FSIS, and therefore industry, approach to nonO157STEC in the near future, unless there is an outbreak which forces FSIS to act. The same can be said about Salmonella in ground beef.
- The Australian industry needs to have the same opportunity to apply chemical interventions that are available to the US industry, without curtailing opportunities for market access.
- Only a few (probably, one) intervention was generally considered to be a CCP, and this will usually be a thermal intervention rather than a chemical one; thermal interventions appear to provide a higher degree of certainty that is not provided by chemical interventions. Using a large volume of water in decontamination processes can enhance their effect, through washing microbes off the carcase
- HACCP plans can be much less complex than Australian HACCP plans and be acceptable to FSIS
- It is important to understand how contamination of a carcase changes during processing steps, including chilling and boning; alongside this is the significance of harvest monitoring (at points along the production line) to measure the increase/decrease in contamination on a carcase. Harvest monitoring could be a useful benchmarking tool for the industry to understand process effectiveness at contamination control.
- Air in chillers and refrigeration systems may be a source of contamination
- If hypochlorite is used for sanitation, it is important to make sure that the pH of the solution is in the right range to obtain the maximum efficacy
- Industry should consider following the US lead and discuss how to share knowledge about food safety practices in a non-competitive manner

A number of questions arose in the course of the study tour, that should be incorporated into industry food safety R&D plans:

- The impact of various chemical interventions on shelf-life and the development of the usual microbes in vacuum packed meat
- The status of the lymph nodes of Australian cattle
- The use of investigation tools for improving process hygiene and sanitation
- The application of various approaches to harvest monitoring as a benchmarking tool for processors
- The use of performance objectives in a process control system
- The significance of air in chillers to carcase contamination

There are opportunities for companies to use Plant Initiated Projects to evaluate new approaches to control of microbes on carcases, primals and trim.

Potential vendors of new technology to the industry should be encouraged to initiate Partnership projects through the MLA Donor Company to ensure that technologies are introduced to the broader industry and evaluated thoroughly under Australian conditions.

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Background

Australian meat is regarded internationally as having a very high standard of safety and the systems used to raise animals, process and transport them are seen by trading partners as being outcomes-based, innovative and effective. However, new demands will always come from consumers, customers, public health organisations and importing countries. Some will be about the safety of product. Other demands will be about suitability or safety-associated attributes of meat production and processing systems.

Recently, the United States FSIS established new rules relating to certain Shiga toxin-producting Escherichia coli (STECs), which requires the application of process control, HACCP, and testing to manage these risks. Processors in the US are applying a range of different interventions to manage risks as well as a range of quality assurance (including testing) responses to address these new requirements. In Australia, there is a need to increase the understanding of the options available to respond to these requirements and respond to presumtive positive and confirmed positive results for STECs.

Project Objectives

The objectives of the project were:

- To update processor companies seeking to define their approaches to HACCP and other activities;
- To update on process control activities – which will inform Australian processing companies and in addition, the process control projects underway to assist industry;
- To expose processors to practical, on site review of quality assurance and intervention approaches in the US;
- To update current information and provide a program approach and extension outputs on the latest practical applications, HACCP activities and interventions; and,
- To inform the direction for the MLA food safety program and intended MLA, AMPC and AMIC future research, extension and engagement or policy approaches.

Methodology

This project addressed the objectives by providing for a *syndicated PIP project* that was directed at examining:

- How US processors and customers are dealing with and addressing the new regulations, with specific focus on HACCP, HACCP review and revised processes.
- What interventions are being applied by processors in the US, particularly what new interventions have been established.
- Determination of what might be applicable to Australian processing companies, nothwithstanding the recognition of comercial decision making for any specific uptake.

The project involved a study program in the USA, designed to examine interventions applied on plant in US and participate in North American Meat Association (NAMA) conference and specific meetings with supply chains using grinding beef.

The composition of the study group and the itinerary of visits/meetings that occurred are listed in Appendix 1.

The schedule of visits and meetings was determined to coincide with the North American Meat Association meeting (Appendix 2). This was known to be a meeting with much practical food safety information directed at small and medium-sized establishments, and had a associated trade show. MLA determined the schedule and the meetings by consulting with contacts within the USA.

A list of general questions to which answers would be sought during the study tour was constructed by MLA, AMIC and AMPC, and then validated and varied after discussion with the members of the study group.

The results of meetings and visits is presented in this report in a question and answer format, based on the questions that were determined prior to embarking on the tour. This was considered more useful than providing notes of each meeting.

Results

A summary of discussions in conferences, meetings and visits is presented in this report in a question and answer format, based on the questions that were determined prior to embarking on the tour. Additional questions have been written to accommodate extra information that was presented during the tour. This was considered more useful than providing notes of each meeting.

Regulations

Current policy direction of FSIS

There are concerns about mechanically tenderised beef and a mandatory labelling rule is in the final stages of clearance. There will be an alert in the label to cook the product adequately. (Such labels are on ground beef, but have little impact.)

Current policy of FSIS for STEC

There does not appear to be hard rules about STEC testing compared to O157. Information will also be provided on approaches to O157 testing because consistency demands that FSIS follow the same direction as for O157.

FSIS is concerned because the prevalence of STEC in veal appears to be higher. ARS is conducting further tests on isolates collected from FSIS testing (including those strains that are not adulterants?). FSIS won't implement testing of ground beef for some time, because additional information is required and certainly won't expand the testing program this financial year.

What actions are being taken when STEC is detected in a lot of trimmings?

- During an FSIS verification test if found positive there will be a Food Safety audit including HACCP (may take 2-3 weeks or more) and 16 follow up samples. The auditor will review all manuals, documents covering several months production, monitor staff who are checking CCPs and pre-operational hygiene and they will produce a number of reports on different aspects of the process. Product must be sent for heat treatment. Due to the new 'hold and test' rules, there should not be a product recall due to FSIS detecting an STEC in trimmings.
- During a routine company test if an STEC is detected product is diverted to an appropriate market (cooking)
- Testing of imported product- Refer to Australian Meat Notice 2012/03

If you test everything then FSIS accepts your negative results (providing you have adequate control over your testing.

FSIS position on O157 v nonO157STEC

O157 is not an index organism (i.e. it does not predict the presence or absence) of other STECs. A negative test result for O157 does not support a claim for absence of other STEC, but it is accepted that interventions for O157 should work for nonO157STEC

FSIS are continuing to look at data coming out of their trim testing program (size of plant, age of animal etc) and are sending isolates to ARS for additional characterisation (presence of virulence markers etc). Some have suggested that the higher prevalence of STEC in veal

trim, may be an artefact because veal is more likely to be processed in smaller establishments with less effective procedures/processes. Therefore the high veal trim result may be because processing has occurred in small establishments. They won't implement ground beef testing for some time because they require more information, probably not this year. It's unlikely that grinders will require testing before FSIS start a ground beef testing program

What is the likely FSIS action if STECs detected in bench trimmings or ground beef?

Not being tested yet – but would expect the same response as for O157.

FSIS are following the same policy as for O157 – multiple implications of a source establishment within a period, will trigger an audit. For Australia, the action should be through the agreed protocol in Australian Meat Notice 2012/03.

What is the overall prevalence? Of each STEC type? In cattle by age? What impact will this have on policy?

IEH clients are finding 0.7% O157 and 0.99% nonO157STEC. This is based on IEH testing methods, which 'confirms' the presence of O157 without isolating the strain.

Some establishments are finding a high prevalence of nonO157 without finding any O157 – the reason why O157 cannot be considered to be an index organism for nonO157STEC.

If products such as veal or other raw ground beef components are found to be positive for STEC, then consumer groups will expect source materials to be tested.

FSIS publishes extensive data on their website (http://www.fsis.usda.gov/science/About_Ecoli_Testing_Program/index.asp) (Appendix 3).

STEC are seen to be more prevalent in veal than in beef, but it is not known to what extent this may be due to the animals and how much to the processor (smaller processors often process veal).

The O genes for the big6 STEC are often found in the faeces of cattle, with the exception of 121 which isn't found as often and O111 which is rare. stx^- and/or eae^- strains are often found.

No one is speculating on how policy might change based on data from testing programs. The only acknowledged circumstance leading to a change in policy would be an outbreak attributable to one of these strains in beef.

Have STEC caused disease in people attributable to consuming meat?

A cluster of *E. coli* O26 illnesses in Maine and New York in 2010 was traced to ground beef produced by Cargill Meat Solutions of Pennsylvania. Cargill has issued a recall for 8,500 pounds of ground beef products for potential *E. coli* O26 contamination. The meat was distributed to BJ's Wholesale Club locations in eight states: New York, Maine, Connecticut, Virginia, New Jersey, New Hampshire, Massachusetts, and Maryland. At least 3 *E. coli* illnesses were connected to the recall - two in Maine and one in New York.

http://www.about-ecoli.com/ecoli_outbreaks/view/cargill-ground-beef-e.-coli-o26-outbreak/

How might policy change if detection is associated with an outbreak?

There is broad acknowledgement by both industry and FSIS, that if a microorganism is associated with an outbreak then that food is adulterated, even if the organism has not been declared to be an adulterant. FSIS will act to ensure that further cases do not occur; they will be forced by public opinion and congressional pressure to take action.

FSIS conducting baseline studies

While FSIS had previously announced that they would conduct a carcase baseline study for nonO157STEC, no mention was made of this baseline. FSIS appear to have a number of higher priorities.

Other FSIS policies in development

FSIS is driven by public health – for example, as expressed in the healthy people goals. Consideration has been given to the foods responsible for Salmonella infections, where FSIS-regulated product is believed to be responsible for 36% of cases. The following FSIS-regulated foods are ranked for contribution to illness:

- Broiler carcase
- Pork
- Deli Meats
- Ground beef

The steps that FSIS will be taking will be

- Emphasis on sanitary dressing (suggested that this will start with poultry – but in March 2013, FSIS released a Notice on veal[1])
- Verification testing (will start conducting Salmonella and STEC verification on trim on one sample later this year)
- Establish new pathogen reduction performance standards
- Provide guidance on the use of indicator organisms
- Step up enforcement action on persistent offenders

Mechanically tenderised and marinaded product will require labelling. This rule is expected before the end of March 2013. It is likely that this will be followed by cooking rules. Pork cooking temperatures were reduced recently, and it is likely that there will be a rule about cooking of mechanically tenderised (or marinaded) product.

HACCP reassessment

How many times can you reassess your HACCP plan?

FSIS expect a HACCP plan to be reassessed if a positive is found in their testing. [JBS/IEH] This follows standard HACCP steps. FSIS have more focus on prevention and control for example: dressing practices (Directive 6410.1 rev1), avoiding cross contamination at hide

[1] http://www.fsis.usda.gov/OPPDE/rdad/FSISNotices/20-13.pdf

removal, process monitoring (hide, fresh carcase, dressed carcase), effective chilling, supportable sampling and analysis, a sound program with clear decision-making based on data.

Often establishments will not change their HACCP plan because it is already considered effective for O157. If the HACCP plan is mature and well-written then what else can be done? Often retraining of staff is suggested as a corrective action.

HACCP plans are reassessed if anything changes – eg testing frequency, sanitary dressing procedures. The purpose is to look at data that has been generated, think about what the results mean, and engage in root cause analysis.

A sample HACCP plan, accepted by FSIS, is to be found in Appendix 5.

Validation

To some degree, establishments have been able to cite scientific papers and reports to support the efficacy of intervention steps (for example, see Appendix 6), but there is a growing expectation that establishments will demonstrate that these interventions work in practice

One company provided a brief description of a validation process for a hot water wash designated as a CCP. They used carcases inoculated with high levels of E. coli strains selected by Iowa State University as representative of O157. Both inoculated and uninoculated carcases were tested before and after treatment for APC and Enterobacteriaceae. Their process showed a 6 log reduction. Since the uninoculated controls averaged 1.2log, they had confidence that that their hot water wash would be effective.

A group of university-based scientists have developed a consensus paper on the validation of antimicrobial interventions (http://www.foodprotection.org/publications/food-protection-trends/article-archive/2013-03validation-of-antimicrobial-interventions-for-small-and-very-small-processors-a-how-to-guide-/). Sometimes laboratory studies can be conducted, or pilot plant studies, or plant-floor studies. Close to the actual production situation is considered to be most acceptable.

Litigation

Legal basis for damages litigation in the USA

US civil law applies the concept of strict liability – if you manufactured it (or in the supply chain, in some states) then you are liable. Unlike Australia, there is no ‘due diligence’ defence in the USA.

Legal cases need to show causality – ie that the product of that manufacturer caused harm. 100% of fault is somewhere in the room – the jury decides where the fault lies. Not easy to claim, for example, that a parent has contributed to death of their child through poor hygiene or inadequate cooking.

Punitive damages may apply if the manufacturer has shown a conscious disregard of a known risk – and you can’t insure against this.

Damages have been awarded in cases where the patient is a secondary case (ie they caught the illness from someone who ate the hamburger)

International laws apply to litigation
An Australian manufacturer can be sued in the US, if they have holdings or assets in the US.

It would be possible to sue in an Australian court, but it may not be cost effective because lawyers may consider that Australian judgements are not high enough.

It is probable that US importers will have contracts with Australian exporters that will include specific indemnities in favour of the importer on product safety and quality issues. Sometimes these clauses of contracts place a very large burden on suppliers.

FSIS policy
Lawyers desire to have no anomalies in regulations, so any microbe in meat that could cause illness should be treated in the same way as O157.

Regulations for other meat-borne pathogens
Lawyers can start litigation for damages if there is any injury – a microorganism doesn't need to be declared an adulterant before a claim can be made. The law allows FSIS to take action against foods that "*may* be injurious to health"

General views of customers (community)
The US consumer has a different perception of risk to Australian consumers – much more concerned about risks associated with foods. Consumers are expecting that raw products such as beef will be as 'ready to eat' as processed products

Food safety is now at the top of consumers' minds when they think about quality

Education programs
Consumer education programs are considered to be of little value and aren't pursued.

Processing

Process

What GMP is used

There needs to be a focus on preventing contamination of carcases:

Hide removal- transfer from hide to carcase

Preventing cross contamination during dressing

Preventing growth during chilling

Two knife systems (cut, sharpen, sterilise), with adequate personnel washing during the changeover

One company has an approach that has three main aspects:

- Process
  - Carcase dressing technique
  - Looking for fold and flaps that would make interventions less effective

    - Carcase separation in chillers
    - Video auditing of dressing techniques, and folds/flaps
- Mechanical interventions
    - Multiple hurdles from hide through to trim and sub-primals
- Monitoring
    - Temperature at carcase surface through hot water wash (3x per shift- multiple points on carcase)
    - Microbiological monitoring
        - Hide on
        - Hide off before any treatment
        - Post-evisceration wash
        - Pre-final hot water wash
        - Post lactic acid spray
        - Going into chiller
        - Coming out of chiller

GMPs applied in Australia are also beginning to be generally applied in the USA. Most processors assess cross contamination very well – and focus on identifying occurrence.

The Verifeye system has generally fallen out of use, particularly the cabinet model. No longer being actively marketed.

Intervention

The following table lists interventions that were observed/discussed during the study tour. :

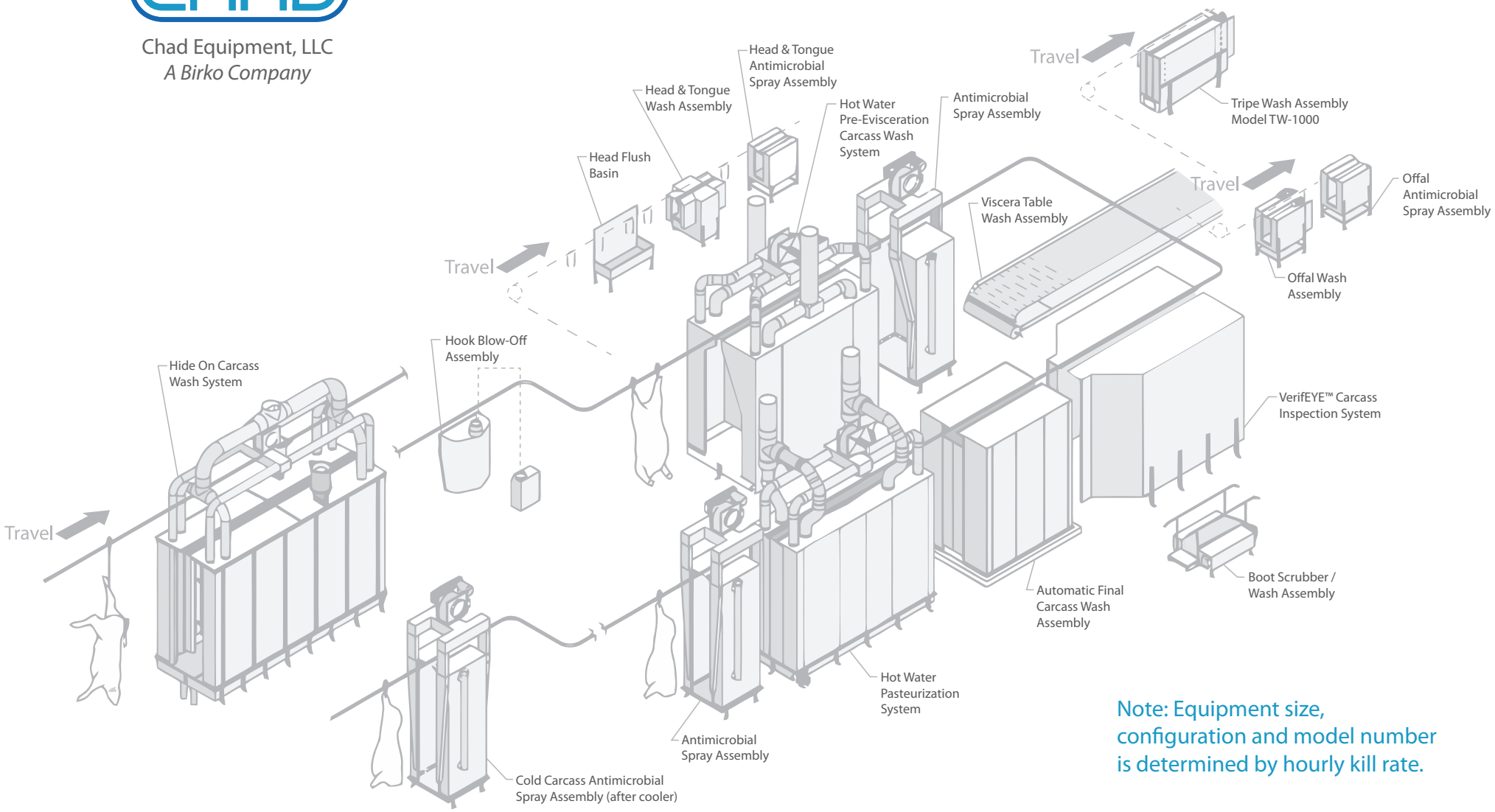
Process step	**Interventions**
Pre stunning	Water wash Chemical
Post stunning	Hot water Chemical De-dagging Steamvac on cutting lines
Legging	Chemical Steamvac
Forequarter	Chemical Steamvac
Post hide removal	Chemical
Hocks	Steam Hot water with vacuum
Pre evisceration	Hot water- 160-165F at carcase surface for 10-15s Chemical
Carcase spitting	Steamvac Chemical
Final carcase	Warm wash for bone dust removal
Final carcase	Hot water Steam Chemical
Pre chilling	Chemical
During chilling	Chemical
Post chilling	Chemical
Primals and trim	Chemical

Heads	Hot Water Chemical

Views of the spray cabinet interior

Cabinet exterior and ancillary tanks/make up equipment
A diagram showing how interventions might be arranged through the process follows:

Chad Equipment, LLC
A Birko Company



Note: Equipment size, configuration and model number is determined by hourly kill rate.

Agents

Water

Hot water is considered to be the best antimicrobial treatment (≥ 76°C at the carcase surface for ≥ 8s). Commonly 160-165F at the carcase surface is used for >10s, with some using 170F for 15 or even 20s. The following notes were from different establishments etc. and so represent different views on hot water decontamination.

Steamvac (Kentmaster is preferred because it delivers a higher temperature to the carcase surface) on cutting lines.

A pre-evisceration wash is used by a number of large establishments with hot water which is recirculated at 160-165F at the surface of the carcase, or more preferably 170F for 15s. Bunging and evisceration occurs after the wash. The head may be on or off.

One processor implemented a hot water decontamination cabinet within the last year. They have seen a four-fold reduction in their rate of positive O157. Non recycled water. Water is 170°F at the point of application for 15 seconds.

Use of water temperature of 180°F at point of contact with carcase for 15s gives a cooked appearance to many parts of the carcase. Initially customers complained, but now they have got used to the idea that this is a food safety intervention. Other processors use slightly less aggressive time/temperature which allows most colour to come back to the carcase during the chilling process.

Final carcase wash – 90°F first wash and then 170°F

Hot water wash cabinet at end of slaughter line with recycled water. The water is filtered to ensure that the nozzles do not block and water is topped up, but there is no control of turbidity, or the turbidity is maintained at <200 NTU. The USDA requirement is that the water is pathogen free. The hot water cabinet is cleaned (CIP_ during sanitation with the recirculation tank being used as a CIP tank. Nozzles need to be replaced once per year in a single shift plant.

Steam- some establishments have direct steam application to whole carcases.

Heads and tongues may be washed manually or in a cabinet. Generally hot water 180F is used, but chemicals may also be used before further work is done.

Many different temperatures and times are used by different processors. The following table in a first estimate of the E. coli death (in logs) for various combinations of time and temperature. Temperature should always be monitored at the carcase surface (Thanks to Tom Ross, University of Tasmania for this table).

Some establishments use multiple temperature sensors on a carcase several times per shift to monitor that the system is operating correctly and the correct temperature is being achieved on the carcase surface. Also there is a low temperature alarm on the water supply. The hot water intervention is defined as a CCP.

Table: expected log decrease in E. coli count with various time and temperature combinations

	dwell time (seconds)				
T °C	5	10	15	20	25
60	0.024764	0.049528	0.074292	0.099056	0.123819
61	0.037968	0.075936	0.113904	0.151872	0.18984
62	0.058213	0.116426	0.174639	0.232851	0.291064
63	0.089252	0.178504	0.267756	0.357009	0.446261
64	0.136842	0.273684	0.410525	0.547367	0.684209
65	0.209806	0.419613	0.629419	0.839226	1.049032
66	0.321676	0.643352	0.965028	1.286704	1.60838
67	0.493195	0.98639	1.479585	1.97278	2.465976
68	0.756169	1.512338	2.268506	3.024675	3.780844
69	1.159361	2.318722	3.478084	4.637445	5.796806
70	1.777537	3.555075	5.332612	7.11015	8.887687
71	2.725328	5.450656	8.175984	10.90131	13.62664
72	4.178484	8.356968	12.53545	16.71394	20.89242
73	6.406469	12.81294	19.21941	25.62588	32.03235
74	9.822426	19.64485	29.46728	39.2897	49.11213
75	15.05978	30.11957	45.17935	60.23914	75.29892
76	23.08972	46.17945	69.26917	92.3589	115.4486
77	35.40126	70.80253	106.2038	141.6051	177.0063

Chemicals

Allow water on a carcase to drain off before applying chemicals to avoid diluting the chemical.

Lactic acid (and Beefxide® – a lactic/citric acid mix that is less corrosive to concrete) and Bromine (BoviBrom®) are considered to be useful. Chemicals such as acidifed sodium chlorite and peroxyacetic acid are considered to be not effective, however, some establishments may rely on a variety of chemicals. 4% is about the maximum concentration that can be used without the carcase becoming brown.

Beefxide® is a mixture of lactic and citric acid that is less corrosive to concrete than lactic acid used alone. If carcases are left on the chain during a break they are sprayed with Beefxide® to minimise growth of bacteria during the processing time http://www.birkocorp.com/beef/beefxide/

BoviBrom® A bromine-based sanitiser from Elanco. Other bromine products are available but this appears to be the leading one. Some other bromine-based products may not work as well. The method of application is considered to be important. This product doesn't cause colour change of meat, nor is it as corrosive as lactic acid. It is suggested that using a product such as this in chillers may have a positive effect on the chiller environment. https://www.elancofoodsolutions.com/products-services/food-safety/

Finalyse® – a bacteriophage product from Elanco. It doesn't seem to be mentioned much, even at the trade show where the supplier was represented. Some processors question the cost-benefit in using it and may have difficulty in accepting the claims made for it. https://www.elancofoodsolutions.com/products-services/food-safety/

Sodium hydroxide may be used for treating hides post bleeding and before any other actions. Use large amounts of solution (>250 L/min at 200psi). It is advised to double shackle the body so that the orientation to the spray jets remains the same. Use air jets to blow excess liquid off the hide before any further operations. Note that this process may not be suitable for kosher or halal slaughter.

Sodium hypochlorite is used in some applications. The pH of water being chlorinated can change depending on temperature of the water as cooler water has a higher pH. Also the lower pH the higher percentage of free available chlorine, the concentration of hypochlorous acid (HOCL) which is a more potent biocide (Low pH in the water will make it more acidic). This means that if we do not have the correct water pH when mixing the chlorine we have taken away the effectiveness of that chlorine when spraying onto carcases (Correct pH Value is around 6.5). Chlorination is an effective and economical solution to the problem of orifice and emitter clogging, due to biological growth. When chlorine is dissolved in water, the chlorine molecules combine with water in a reaction called hydrolysis. The hydrolysis reaction produces hypochlorous acid (HOCl). Following this reaction, hypochlorous acid then undergoes an ionization reaction to produce hypochlorite: Hypochlorous acid (HOCl) and hypochlorite (OCl-), which are together referred to as "free available chlorine", coexist in an equilibrium relationship which is influenced by temperature and pH. Where water is acidic (low pH) the above equilibrium shifts to the left and results in a high percentage of the free available chlorine being in the form of HOCl. Where the water is basic (high pH), a high percentage of the free available chlorine is in the form OCl- The efficiency of Hypochlorous acid in killing microorganism is about 40 to 80 times greater than hypochlorite. The effectiveness of chlorination is highly dependent upon the pH of the water source, quantity of bacteria, algae and other organic matter. Thus, water having a low pH will result in a high concentration of HOCl which is the more potent biocide. At pH 8, only about 22% of the chlorine will be in the active HOCl form, at pH 7, 73% will be in the HOCl form, and at pH 6, about 96% of the chlorine will be in the HOCl form (Nakayama and Bucks, 1986). If water pH is above 7.5, may be necessary to add acid to lower the water pH. Beware as a low pH under a value of 7 can cause water to go acidic. High pH water in spray chilling could cause premature corrosion & rust in chillers. For further information see Appendix 4.

Beef Products Inc (BPI) claim that the best intervention by far is ammonium hydroxide. In addition to its use on carcases, it can be used in chillers and in CIP of refrigeration coils. It is approved by FSIS but no one is using ammonium hydroxide in spray chilling.

Physical methods

UV light of appropriate wavelengths can induce oxidation and produce hydrogen peroxide and ozone in quantities that can be antimicrobial. Application of UV can be intense such as in using on a carcase or on (sub)primals, or can be of lower intensity,

for example for sanitation of carcases during the time that they are in chillers. It may be possible to get a 5 log reduction in the chiller which would allow the claim of 'pasteurisation' to be made. Puradigm is developing these approaches with the assistance of Jim Marsden at Kansas State University.

Preharvest

Little mention made of vaccines, even when direct fed microbials (Probiotics) were being mentioned. It is understood that Bioniche may have not proceeded with regulatory approval in the US, and the Epitopix vaccine failed to show significant impact when tested at a time when the natural prevalence of O157 was low in untreated animals.

How do you deal with dirty cattle? What issues need to be addressed (eg animal welfare)

- Spray live cattle – water to settle dust, hypochlorite in water used to spray cattle prior to knocking
- Removal of dags - dedagger, air knives, brush, brisket saw
- Steam vac cutting lines, hot water rinsing of cutting lines (manual)
- Finalyse – bacteriophage product for application to hides

What combinations of interventions are used?

Every establishment, it seems, has its own combination of interventions, built up over time, and with no inclination to take anything out if the system is working. The table shows three possible intervention scenarios

Table: Examples of intervention combinations in 3 slaughter plans

A	B	C
Wash cattle – hypochlorite	Wash cattle post sticking	Soap wash for hide of live cattle
180°F water applied to cutting lines of the hide – cooled down with chlorinated water	Lactic acid on cutting lines just before opening cuts	Steam vac of hide cutting lines before opening cuts
170°F 15s water in a spray cabinet	Steam vac on cutting lines as hide is removed	Steam vac or steam 'hood' on hocks
50ppm chlorine applied in chiller	170°F water pre-evisceration wash - ~140°F on contact with carcass <15s (1.7 log reduction in E. coli)	Pre-evisceration wash180F at carcase surface for 12s
Beefxide® (lactic/citric) mix applied just before boning	204°F hot water (180°F at point of contact with carcase), 15s. (2.0 log reduction in E. coli)	End of line 180F at carcase surface for >12s
200ppm Quat sanitiser on cutting boards at breaks	Beefxide® just before entering the boning room	Cold water bromine wash as soon as carcase has dripped excess water
	Peracetic acid spray to top and bottom of subprimals	Lactic acid spray cabinet plus manual lactic acid wash

	just before packing	just before chiller
	Beefxide® wash on heads and flush oral cavity with 180°F water	Bovibrom® in spray chill water
		5% lactic acid as carcases enter boning room
		Lactic acid of primals/subprimals/trim before packing
		Beefxide® just before grinding

Quality Assurance

Is carcase E. coli test data used. If so how?

ESAM type testing is performed because FSIS require it, and it isn't useful and isn't looked at.

How do you manage consistency / training in the workforce?

Some companies have video monitoring on critical jobs which allows them to review practices – especially if there is a problem. Videos are also used in training.

At one company staff are paid an incentive for maintaining good micro results for a certain number of days – this encourages continued compliance and team members reinforcing good behaviour. In the case of a problem everyone is asked for suggestions about how to improve.

What real time measures or feedback data do you most rely on – and how do you sort lots accordingly?

Didn't see any physical visual observation of carcases.

Some establishments rely on O157 or STEC testing and rapid turnaround of results. Others use an IEH test called Meat Process Control (MPC) and will make disposition decisions based on these results. Often 'shoulder' lots -1 before and after- the lot in which O157/STEC were detected will be sent to cooking. There are event day programs for multiple positives in a day.

If you were starting again, what controls would you implement in your plant?

Space has been an issue in implementing interventions – sometimes determining what interventions can be implemented. Most establishments seem to have a shortage of chiller space.

Once an intervention is implemented it is very difficult to decide to eliminate it, therefore most establishments have intervention on top of intervention.

How is traceback and recall achieved

Majority of establishments seem to be using single combo lots. Test results may be turned around in 12 hours. Product may be despatched, but held under company control until results are obtained – ie product may be returned to the establishment rather than being delivered to the customer.

How is the process monitored?

IEH suggest three steps:

- Monitor practices
- Audit carcases for deep cuts, folds and flaps that may make spray interventions less effective
- Harvest monitoring: transfer of microbes from hide to carcase and reduction in counts on carcase through interventions. http://www.iehinc.com/meatmonitoring.html

This approach is used by some companies as their framework for monitoring process.

One company performs more extensive monitoring (testing the same carcase through the process as much as possible), using APC (TVC) and Enterobacteriaceae as indicators:

- Hide on
- Hide off before any treatment
- Post-evisceration wash
- Pre-final hot water wash
- Post lactic acid spray
- Going into chiller
- Coming out of chiller

Use of performance objectives

One company is using the concept of performance objective (PO). In this case, a performance objectives is the maximum count of an indicator organism (usually, a performance objective is only used for a hazard) that is acceptable at a particular point in the process. It is accepted that a PO is a 'signpost' along the supply chain that eventually contributes to meeting food safety objectives, and thus providing consumers with an acceptable level of protection.

This company uses Aerobic Plate Count (APC, approximately equivalent to TVC), as an indicator and has set the PO as

- ≤ log 2.per cm^2 on the carcase immediately after hide removal
- ≤ log 1.5.per cm^2 on the carcase immediately before the final hot water wash
- ≤ log 1.0.per cm^2 on the carcase immediately post chill

The method for using these POs was not discussed, but clearly they set limits that direct plant personnel to give attention to particular parts of the process, and to investigation, should these numbers be exceeded. Achieving these POs suggests that the establishment has determined that subsequent interventions will be able to deal with this level of contamination and provide a high level of product safety.

What is your event day program?

Event day actions should occur if there are more than 5% positives on a single day (or even within part of the day)

Various responses:

- implement tailored responses if finding more than 2 positive combos in a day.
- If a single positive test result is obtained 1 hour before, and 2 hours after, it is sent for cooking – most of it with a clear CofA – or to a customer who is known to cook
- On an event day – all product is sent for cooking. Also N60 testing is applied to sub/primals with each hour of processing being considered a lot – and the same criteria are applied to sending for cooking.

FSIS position

FSIS expect 5% positive to be considered an event day, but some establishments set a lower limit, consistent with a process control approach.

FSIS conduct an FSA audit which includes looking at HACCP reassessment and 16 follow-up samples.

Product

Does the plant have restrictions on exporting decontaminated product to any destination?

No problems.

No problems with Japan.

No problems if it is an intervention that is approved in the US and not banned in the importing country.

Probably specific permission has not been obtained; it is just not considered. A common view is that these chemicals are processing aids and therefore do not need to be labelled.

Singapore has recently issued a list of chemicals that may be used on carcases[2].

How is shelf life affected by decontamination?

There is a suggestion that spray chilling may cause problems with shelf life because carcases are maintained at a high temperature for longer with high water activity at the surface. But the practice of filling chillers and having touching carcases may also contribute to problems.

No one seems to know, or have asked about how decontamination processes affect shelf-life.

What are the common spoilage problems?

Discolouration of fat in vacuum packed primals and large volumes of purge.

Sampling and testing

Different establishments are testing differently for nonO157STEC. IEH has established the following options:

[2] http://www.ava.gov.sg/NR/rdonlyres/52A066C1-7026-49F9-9B3B-A12F1291D922/25355/circular_AntimicrobialSpray.pdf

- Align with O157 (may include primal/subprimal testing) (some IEH clients)
- Only test trim lots
- Quarterly
- Only O157 positive lots and maybe those immediately before and after (not supportable)
- 1 day/week (some clients – provides some data for decision-making)
- Whenever FSIS tests (1-4 times/month) (followed by most IEH clients)

Others confirm that few establishments are testing all samples. Some are testing 1 sample per day, only test O157 positive product, because that is being cooked anyway, or only testing event days.

Establishments are likely to start testing either because the grinders start testing (which in turn will be because FSIS starts testing ground beef), or when the tests yield acceptably unequivocal results in the same time and at the same price as O157 tests.

How is sampling conducted?

We saw two establishments using the IEH N-60plus sampler, which is a cutting device on a drill, which stores product in the core of the cutting part until all sampling is completed. A single combo bin is sampled at a minimum of 5 points, with the sampling device shaving off a very thin slice. Around 200g is sampled, but this is considered to be equivalent to taking 375g of manually sliced pieces. They validate by comparing standard and N-60plus sampling for micro counts (APC, Enterobacteriaceae and E. coli) and mass obtained. FSIS have issued a letter of no objection see http://www.iehinc.com/n60.htm .

Where is sampling conducted?

Single combo bins are tested at many establishments either by choice or as a requirement of the school lunch program. The claim of IEH is that single combo testing:

- Detects more lots with O157
- Costs 30% more than 5 combo-bin testing
- Results in less product being sent to cooking – on average only 1.6 combo bins out of 5 are found positive.

Do you test carcases for STEC?

Testing for O157 can be used to verify the effectiveness of food safety procedures that should also be effective against nonO157STEC providing that enough testing is done to demonstrate the effectiveness of the system – but this does not demonstrate control of nonO157STEC (because you don't know the concentration and sources of nonO157STEC that are independent of O157).

Are any indicators used?

IEH promotes an idea for testing that is focussed on measuring indicators of carcase contamination rather than pathogens. There is a PCR test that simultaneously measures markers for E. coli, EHEC, Salmonella, Listeria, Enterobacteriaceae, anaerobes, Pseudomonas. The primers may not be precise for detecting pathogens of concern – therefore there is no negative regulatory implication. Some pathogens have two possible

bands. There is a scoring system of 1 point per band – but 10 points if both bands are present for a pathogen. The client is only provided with the score. They are then able to choose to send product to cooking if the score is over 10. The results can be monitored over time in a process control chart.

Using the scoring system, all staff are paid an incentive for maintaining low scores for a certain number of days – this encourages continued compliance and team members reinforcing good behaviour. In the case of a problem everyone is asked for suggestions about how to improve.

See under the heading 'Quality Assurance' for a description of the use of indictor organisms and performance objectives to monitor process.

Some companies test all trim for Salmonella, APC, generic E. coli, and coliforms in addition to O157.

How is testing conducted? Turnaround times?

Many laboratories work 24h/day. Tests are set up and results read continuously through the day. With 8 hour enrichment, it takes about 10 hours to provide a test result.

How do you make decision on lots not to be shipped for grinding? Where do these lots go? At what cost?

Processors may send product to cooking from the positive lot and the lots either side of the lot that tested positive, even if they have a negative result. Lots sent to cooking may be sold more cheaply, even if the cooker is a regular customer, because they still want to see a certificate of analysis for the product. Product from either side (shoulder lots) may be sold at usual prices.

Decisions made from process monitoring

Lots may be sent to cooking based on the IEH process monitoring test (test described as an 'indicator' test above) without having a definitive test result. This is seen as being cautious from a public health point of view and doesn't cause problems with the regulator because it isn't accepted as a pathogen test.

Developments in testing

Test kit manufacturers are conducting additional R&D to get screening rates lower, so that a greater proportion of potential positives are confirmed. This will make testing more useful and cost effective.

Roka are developing a system that attempts to identify E. coli that carry enough virulence genes to be of public health concern. This could be a helpful test, but is likely to identify strains broader than the top 7 strains of concern to FSIS. It's not known how much effort is going in this direction compared to Salmonella and Listeria test methods (note: MLA is working with Neogen and CSIRO to evaluate this test under Australian conditions).

Neogen has developed a method that can be used for confirmation. FSIS has issued a letter of no objection for this as a confirmation test. It gives 24 hour turnaround at around $35 per

sample (note: MLA is working with Neogen and CSIRO to evaluate this test under Australian conditions).

Pall Genesystems are working on a new development, linking O types with the variants of eae and H genes to better predict whether all of the necessary genes are present in a single cell within an enrichment broth.

Grinding

What controls do you expect your suppliers to have in place?

Suppliers provide a letter of guarantee that systems are in place to prevent adulteration and then supply Certificate of Analysis, from a lab complying with FSIS guidelines, using acceptable (N-60) sampling and appropriate methods. Grinders want to know about validation of interventions, the scope of audits carried out, reassessment for STECs, program for high event period, meaningful ongoing verification.

Some grinders have different requirements for different countries. They say whether the hazard is likely to occur. Grinders can have fewer interventions when using Australian product because of our historically low level of O157.

Specifications for STEC have not been implemented, but when FSIS start to test ground beef this position will be reassessed.

How are batches of meat for grinding assembled. Do you limit the number of source plants in a batch.

Up to five batches of beef trim may be used in a grind to manage the fat content. Therefore, it is usually not possible to follow back with any certainty.

If there is a positive lot, how is that defined? How much product is rejected? What traceback can you do?

One grinder collects patties from the line every 15 minutes and composited samples are tested for each 2 hour period. If a composite is positive for O157 then the 2 hours before and after a sent for cooking (at a discount price) and the positive product goes to landfill. All the individual retention samples are then tested for the entire day (20 hours x 4 per hour- 125g samples) to get an understanding of the contamination on that day. This large grinder has had only 14 positive samples in the past 10 years.

Companies do their best to identify possible sources of contamination, and may use records of indicator organisms to suggest where contamination may have come from. They ask all implicated suppliers to review their records for any abnormalities that may signal a problem, and expect a report. In part, this is to cover their regulatory obligation. They try to work with suppliers to iron out problems.

Industry-wide policy

Do companies share knowledge on hygienic processing. If so how.

Many companies share experience through the Beef Industry Food Safety council (BIFSCo) that publishes best practice guides as well as holding an annual conference, and distributing summaries of scientific literature and presentations. http://www.bifsco.org/

Particularly medium to small processors, share knowledge and information, provide each other with advice etc. Amongst the larger processors, there is also cooperation with planned visits between processor QA personnel – no operations people, no notes, no photographs.

What support services does your organisation provide to processors?

The North American Meat Association has launched a Research and Outreach Foundation and expecting to obtained donations that will allow them to fund processor-oriented food safety research. They will work with the National Cattlemen's Beef Association, who fund pre-harvest research.

The American Meat Institute Foundation also funds research work, particularly on processed meat products.

The three foundation organisations (AMI, NAMA and NCBA) get together once a year to discuss priorities and progress on carious research projects.

An issue for the future: *Salmonella*

The issue of Salmonella in ground beef – originating from beef trim, was a hot topic at the NAMA meeting. The issue is that multi-antibiotic resistant Salmonella of certain serotypes that have caused severe disease in the USA.

The Center for Science in the Public Interest (CSPI) have petitioned FSIS for an 'interpretive rule', which will provide no opportunity for consultation or discussion and becomes effective when the agency publishes the interpretation. Some believe that FSIS is trying to ignore the CSPI petition.

FSIS appears to be willing to let the industry take some initiative in this area without making regulation. FSIS is looking for a way that the industry can collect data without it impacting negatively on food safety assessments. FSIS acknowledges that Salmonella strains vary in their ability to cause illness in humans.

If there is an outbreak due to Salmonella in ground beef then FSIS will be forced to act.

There is a reasonable amount of work going on by Guy Loneragan at Texas Tech University.

Over the past 10 years there has been a significant reduction in the prevalence of E. coli O157 in beef trim, and also in E. coli O157 in ground beef, but there has been very little reduction in Salmonella. S. Montevideo is the most common serotype in ground beef year after year and is also in the top few serotypes isolated from human infections.

While E. coli was seen as being a processor issue because E. coli moves from the hide to carcase by the actions of processors, Salmonella is seen as being a producer issue because the Salmonella is in the lymph nodes (and in the meat) prior to the animal entering the slaughter establishment.

Additional pressure may come on the beef industry to deal with this issue because of the development of new models for attributing foodborne disease to different foods. In the old attribution model beef was considered to be responsible for 2.8% of human salmonellosis but the new attribution model will suggest that beef is responsible for 9.8%. No one knows how FSIS might respond to this in their priority setting.

In a Texas Tech University (TTU) study over 2012 calendar year, 6% of subiliac lymph nodes were positive for Salmonella (Brichta Harhay, 2008 method). Differences were observed regionally, seasonally and in cattle type. There was a positive correlation between faecal and LN prevalence. Some lymph nodes may contain $>10^7$ cfu Salmonella/lymph node without these nodes appearing, macroscopically different to 'normal' nodes. S; Montevideo was predominant in both lymph nodes and faeces, whereas, S. cerro was found quite often in faeces but never in lymph nodes. Feedlot cattle were found to have a less variety of serotypes in their lymph nodes, but cull cows were more likely to have Salmonella strains considered to be virulent for humans. This is clearly a complex situation that requires further testing and analysis.

A separate study, also at TTU very high prevalence of Salmonella in subiliac, mandibular, mesenteric lymph nodes were observed, but in a low percentage of mediastinal lymph nodes.

There is a hypothesis that cattle are infected transdermally, with biting flies, skin lesions, or foot rot allowing the entry of Salmonella from hide (faecally contaminated) into the animal, and thus lymph nodes. Some experimental work has shown that Salmonella inoculated in the hock can be transmitted as far as the prescapular/subiliac lymph node, but not further than this.

Probiotics (Bovamine; NP51) at high dose (10^9/head/day) can lead to a reduction in prevalence and concentration of Salmonella in lymph nodes.

There is still a long way to go in understanding the ecology and 'pathology' of Salmonella in lymph nodes, as well as effective interventions that will reduce the prevalence of Salmonella in ground beef.

Discussion / Conclusion

In a tour such as this, there are many discoveries which differ from person to person, only some of which are easily communicated as explicit knowledge. At the end of the tour the group reviewed the developing report and selected and collated the following important messages for the broad Australian processing sector:

- There is unlikely to be any change in FSIS, and therefore industry, approach to nonO157STEC in the near future, unless there is an outbreak which forces FSIS to act. The same can be said about Salmonella in ground beef.
- The Australian industry needs to have the same opportunity to apply chemical interventions that are available to the US industry, without curtailing opportunities for market access.
- Only a few (probably, one) intervention was generally considered to be a CCP, and this will usually be a thermal intervention rather than a chemical one; thermal interventions appear to provide a higher degree of certainty that is not provided by chemical interventions. Using a large volume of water in decontamination processes can enhance their effect, through washing microbes off the carcase.
- HACCP plans can be much less complex than Australian HACCP plans and be acceptable to FSIS.
- It is important to understand how contamination of a carcase changes during processing steps, including chilling and boning; alongside this is the significance of harvest monitoring (at points along the production line) to measure the increase/decrease in contamination on a carcase. Harvest monitoring could be a useful benchmarking tool for the industry to understand process effectiveness at contamination control.
- Air in chillers and refrigeration systems may be a source of contamination.
- If hypochlorite is used for sanitation, it is important to make sure that the pH of the solution is in the right range to obtain the maximum efficacy.
- Industry should consider following the US lead and discuss how to share knowledge about food safety practices in a non-competitive manner.

A number of questions arose in the course of the study tour, that should be incorporated into industry food safety R&D plans:

- The impact of various chemical interventions on shelf-life and the development of the usual microbes in vacuum packed meat
- The status of the lymph nodes of Australian cattle
- The use of investigation tools for improving process hygiene and sanitation
- The application of various approaches to harvest monitoring as a benchmarking tool for processors
- The use of performance objectives in a process control system
- The significance of air in chillers to carcase contamination

There are opportunities for companies to use Plant Initiated Projects to evaluate new approaches to control of microbes on carcases, primals and trim.

Potential vendors of new technology to the industry should be encouraged to initiate Partnership projects through the MLA Donor Company to ensure that technologies are introduced to the broader industry and evaluated thoroughly under Australian conditions.

The objectives of this project have been met as follows:

- To update processor companies seeking to define their approaches to HACCP and other activities;
    - Through conducting the tour, and producing this report
- To update on process control activities – which will inform Australian processing companies and in addition, the process control projects underway to assist industry;
    - Through conducting the tour, producing this report, and making recommendations for further R&D
- To expose processors to practical, on site review of quality assurance and intervention approaches in the US;
    - Through the tour which visited five beef processing facilities
- To update current information and provide a program approach and extension outputs on the latest practical applications, HACCP activities and interventions;
    - The information in this report, and collateral information collected, provides a basis for the development of information resources
- To inform the direction for the MLA food safety program and intended MLA, AMPC and AMIC future research, extension and engagement or policy approaches.
    - Recommendations made here for further R&D

Appendices

Appendix 1 Tour members and itinerary

Tour members

Tony Beadle	Midfield International
Robert Cox	Borthwicks (Nippon)
Shane Gee	Teys Australia
Tracy Hemsworth	Teys Australia
Ian Jenson	Meat & Livestock Australia
Trevor Moore	Northern Cooperative Meat Company
Peter Moore	Kilcoy Pastoral
Belinda Spiers	JBS

Itinerary

February 2013

Day	Date	Time	NAMA conference	meetings	plant tour
W	6			IEH Laboratories and Consulting - Seattle	
		0830		FSIS STEC Policy - Domestic and Imported Products –Ken Petersen	
		0930		Pathogenic E. coli in beef processing plants: Prevalence and controls. Mohammad Koohmaraie	
		1030		break	
		1100		Q & A about the two presentations	
		1200		lunch	
		1400		Bill Marler - food safety litigation attorney	
		1500		Testing platforms for testing pathogens in beef. Mansour Samadpour	
		1600		Tour of IEH Laboratories	
T	7	0930-1200			Schenk Packing, Stanwood WA
F	8	0930-1300			Washington Beef, Toppenish WA
S	10	1545-1645	marketing emerging trends forum		
		1700-1845	opening session - keynote on economic crisis		

M	11	0815-0915	bullet session	
		0930-1030	science and government roundtable	
		1045-1145	beef forum	
		1200-1700		
		1400-1600		
		1400-1430	exhibit hall	Bo Reagan - NCBA
		1430-1515		Dane Bernard - Keystone
		1515-1600		Laurie Bryant - MICA
		1830-2115	networking reception and dinner	
		0645-0800	breakfast- retailing of the future	
		0800-1300		
T	12	0900-1000	exhibit hall incl lunch	Wendy Warren- AEGIS
		1000-1100		Eldon Roth - BPI
		1300-1430		Jim Marsden - Kansas State University Jody Allgood - Puradigm
		1430-1530	food safety forum	
		1545-1645	food safety committee	
		1700-1830		Betsy Booren - American Meat Institute
W	13	0730-0830	legislative and regulatory	
		1600-1730		Mandy Carr - NCBA

T	14	0800		Cargill - Fort Morgan (2 people) CO
		1400-1630	Mark Swanson - Birko Chad	
F	15	0830-1300		JBS – Greeley CO
M	18	1000-1600	Guy Loneragan, Mindy Brashears, Chance Brooks - Texas Tech University	
T	19	1000-1300		Caviness Beef Packers, Hereford TX
W	20	0800-1000		Cargill Meat Solutions - Fort Worth TX

Appendix 2 NAMA conference program

North American Meat Association

MeatXpo'13

SUNDAY, FEBRUARY 10
6:30am – 5:00pm Registration, *Registration Desk 1*
7:00am – 2:00pm Golf Tournament, *off site (ticket required) meet at the North Valet Entrance*
7:45am – 12:00pm Road Biking Adventure, off site *(ticket required) meet at the North Valet Entrance*
8:00am – 8:00pm Exhibitor Move- In, *Events Center*
2:30pm – 3:30pm Associate & Allied Advisory Committee, *Martinique*
Education Committee, *St. Kitts*
Membership Committee, *Barbados*
3:45pm – 4:45pm Marketing Emerging Trends Forum, *St. Croix A&B*
5:00pm – 6:45pm NGA Opening Keynote Session (NAMA Invited), *Grand Ballroom*
7:00pm – 8:00pm NAMA PAC Social, *St. Croix Patio*

MONDAY, FEBRUARY 11
6:00am – 7:00am NGA's Fun Run, *Mirage Course*
6:30am – 7:00am Coffee Service, *Calypso Court*
6:30am – 5:00pm Registration, *Registration Desk 1*
6:45am – 8:00am Issues, Answers, Actions Breakfast, *Bermuda*
8:00am – 11:00am Exhibitor Move-In (by appt), *Events Center*
8:15am – 9:15am General Session (Bullet), *St. Croix A&B*
9:30am – 10:30am Science & Government Roundtable Seminar, *Ballroom C*
Trade & Marketing Roundtable Seminar, *Ballroom D*
Business & Industry Roundtable Seminar, *Ballroom B*
10:30am – 10:45am Coffee Service, *Calypso Court*
10:45am – 11:45am Animal Protein Forum, *St. Croix A&B*
12:00pm – 5:00pm EXHIBIT HALL OPEN
12:00pm – 5:00pm ….Consultants Corner
12:15pm – 1:15pm ….University Students Cook-off/luncheon
1:45pm – 2:15pm ….Specialty Meeting – Hold and Test, Mandatory Recall Programs
2:45pm – 3:15pm ….Specialty Meeting – Regulatory Services
4:00pm – 4:30pm ….Wine Tasting
5:15pm – 6:15pm Marketing Emerging Trends Committee, *Ballroom C*
Processed Meat Committee, *Ballroom D*
Animal Protein Committee, *Ballroom B*
6:30pm – 7:00pm Welcome Networking Reception, *Ballroom Foyer*
7:15pm – 9:15pm Welcome Networking Dinner, *Bermuda*

TUESDAY, FEBRUARY 12
6:30am – 7:00am Coffee Service, *Calypso Court*
6:30am – 5:00pm Registration, *Registration Desk 1*
6:45am – 8:00am NGA/NAMA Super Breakfast - *Meat Department of the Future, Grand Ballroom*
8:00am – 1:00pm EXHIBIT HALL OPEN
8:00am – 1:00pm ….Consultants Corner
9:00am – 9:30am ….Specialty Meeting – Patent Law
10:00am – 10:30am ….Specialty Meeting – Canadian Beef Inc.
11:45am – 12:00pm ….Cash Giveaway
12:15pm – 1:00pm ….Exhibit Hall Luncheon
9:30am – 3:00pm Spouse Tour, *off site (ticket required) meet at the North Valet Entrance*
1:00pm – 10:00pm Exhibitor Move-out, *Events Center*
1:15pm – 2:15pm Workplace Issues Forum, *Ballroom C&D*
2:15pm – 2:30pm Coffee Service, *Calypso Court*
2:30pm – 3:30pm Food Safety Inspection Forum, *St. Croix A&B*
3:45pm – 4:45pm Food Safety Committee, *Ballroom B*
Intellectual Properties Committee, *St. Kitts*
Future Leaders Group Committee, *Martinique*
5:00pm – 6:00pm Workplace Issues Committee, *Barbados*
6:30pm – 9:30pm Annual Gourmet Sausagefest *(ticket required), Montego*

WEDNESDAY, FEBRUARY 13
6:30am – 7:30am Coffee Service, *Calypso Court*
6:30am – 12:00pm Registration, *Registration, Desk 1*
7:30am – 8:30am Legislative & Regulatory Update, *St. Croix A&B*
8:00am – 12:00pm Exhibitor Move-out, *Events Center*

8:45am – 10:15am Board of Directors Meeting, *Ballroom F*
10:15am – 10:45am break
10:45am – 12:00pm Annual Meeting, *St. Croix A&B*

Appendix 3 FSIS data for STEC testing

FSIS publishes extensive data on their website (http://www.fsis.usda.gov/science/About_Ecoli_Testing_Program/index.asp) The following information was downloaded from the FSIS website for 2012:

Results from Analysis of Raw Ground Beef / Veal Component Samples for *E. coli* O157:H7: Calendar Year 2012[1]

Raw Ground Beef Components (RGBC)

Source[2]	As of Dec 31, 2012					
	Federal Plants					Import
	Trim Verification	Follow-up to RGB Positive at Supplier	Follow-up to RGBC Positive	Other RGBC Verification	Bench Trim Verification	Verification / Follow-up
Beef	0.53%[3] (12/2,263)[4]	0.00% (0/208)	0.66% (3/455)	1.04% (3/288)	0.00% (0/797)	1.79% (4/223)
Veal	7.89% (3/38)	10.00% (1/10)	1.67% (2/120)	0.00% (0/5)	0.00% (0/33)	0.00% (0/0)
Mixed[5]	0.00% (0/0)	0.00% (0/0)	0.00% (0/0)	0.00% (0/0)	0.00% (0/0)	0.00% (0/0)
Unknown[6]	0.00% (0/1)	0.00% (0/0)	0.00% (0/1)	0.00% (0/0)	0.00% (0/0)	0.77% (4/518)
TOTAL	0.65% (15/2,302)	0.46% (1/218)	0.87% (5/576)	1.02% (3/293)	0.00% (0/830)	1.08% (8/741)

[1] Results are posted according to the sample analysis completion date.
[2] FSIS uses product labeling for the purpose of identifying whether the sample source is beef or veal.
[3] Percent Positive
[4] (Number positive/number analyzed)
[5] Mixed source is composed of both beef and veal products.
[6] Inspector did not answer the question on the sampling form.

Results from Analysis of Raw Ground Beef / Veal Component Samples for STECs[1]

Raw Ground Beef Components (RGBC)[2]

Source[3]	Serotype	As of Dec 31, 2012			
		Federal Plants			Import
		Trim Verification	Follow-up to RGB Positive at Supplier	Follow-up to RGBC Positive	Verification / Follow-up
Beef	**O157:H7**	0.53% [4] (12/2,263)[5]	0.00% (0/208)	0.66% (3/455)	1.79% (4/223)

	Total non-O157 STECs	0.91% (14/1,533)	1.03% (1/97)	1.74% (6/345)	0.00% (0/212)
	O26	5	1	4	0
	O45	0	0	0	0
	O103	7	0	2	0
	O111	2	0	0	0
	O121	0	0	0	0
	O145	0	0	0	0
Veal	**O157:H7**	7.89% (3/38)	10.00% (1/10)	1.67% (2/120)	0.00% (0/0)
	Total non-O157 STECs	13.04% (3/23)	0.00% (0/0)	19.33% (23/119)	0.00% (0/0)
	O26	0	0	4	0
	O45	1	0	2	0
	O103	0	0	14	0
	O111	1	0	1	0
	O121	0	0	0	0
	O145	1	0	2	0
Mixed[6]	**O157:H7**	0.00% (0/0)	0.00% (0/0)	0.00% (0/0)	0.00% (0/0)
	Total non-O157 STECs	0.00% (0/0)	0.00% (0/0)	0.00% (0/0)	0.00% (0/0)
	O26	0	0	0	0
	O45	0	0	0	0
	O103	0	0	0	0

	O111	0	0	0	0
	O121	0	0	0	0
	O145	0	0	0	0
Unknown[7]	**O157:H7**	0.00% (0/1)	0.00% (0/0)	0.00% (0/1)	0.77% (4/518)
	Total non-O157 STECs	0.00% (0/2)	0.00% (0/0)	0.00% (0/0)	0.00% (0/37)
	O26	0	0	0	0
	O45	0	0	0	0
	O103	0	0	0	0
	O111	0	0	0	0
	O121	0	0	0	0
	O145	0	0	0	0
Total	**O157:H7**	0.65% (15/2,302)	0.46% (1/218)	0.87% (5/576)	1.08% (8/741)
	Total non-O157 STECs	1.09% (17/1,558)	1.03% (1/97)	6.25% (29/464)	0.00% (0/249)
	O26	5	1	8	0
	O45	1	0	2	0
	O103	7	0	16	0
	O111	3	0	1	0
	O121	0	0	0	0
	O145	1	0	2	0

[1] Results are posted according to the sample analysis completion date.

[2] FSIS test results for the 6 target non-O157 STECs appear for only samples of beef manufacturing trimmings from cattle slaughtered on-site on or after June 4, 2012. At this time, only beef

manufacturing trimmings and not components (such as bench trim, or other components of ground beef such as cheek meat and head meat) are eligible for testing for non-O157 STEC, as well as *E. coli* O157:H7.

[3] FSIS uses product labeling for the purpose of identifying whether the sample source is beef or veal.

[4] Percent Positive

[5] (Number positive/number analyzed). Postives and Percent Positives on this table include all serogroups tested and do not reflect the total number of individual positive samples on the Individual Positives and the non-O157 STEC YTD tables. This is due to the possibility that one sample may be positive for multiple serogroups.

[6] Mixed source is composed of both beef and veal products.

[7] Inspector did not answer the question on the sampling form.

In 2012, the following detections were made for imported product:

Table 2. Raw Ground Beef Components (RGBC) Analyzed for Target STECs, Calendar Year 2012[3]

Sample Source4	Collection Date	Target STECs	Where Collected
Import	Sep 7, 2012	O157:H7	Canada
Import	Sep 7, 2012	O157:H7	Canada
Import	Aug 30, 2012	O157:H7	Canada
Import	Jun 14, 2012	O157:H7	Australia
Import	May 30, 2012	O157:H7	Australia
Import	May 4, 2012	O157:H7	Uruguay
Import	Jan 24, 2012	O157:H7	Australia
Import	Jan 10, 2012	O157:H7	Australia

[3]**Positives** on this table are not equivalent to positives on the Summary Year-to-Date or Serogroups tables because one sample may be positive for more than one serogroup.

Appendix 4 Hypochlorite effectiveness

CHLORINE EFFICACY

Michel van Schaik, Aquaox LLC

Introduction

Chlorine is one of the most commonly used disinfectants for water disinfection. Chlorine can be applied for the deactivation of most microorganisms and it is relatively cheap. Chlorine is commercially available as gaseous Chlorine (CL_2) and as Sodium Hypochlorite liquid or powder (NaOCL).

Both gaseous Chlorine (CL_2) and Sodium Hypochlorite (NaOCL) have very limited disinfecting properties. It is the formation of chlorine by-products such as Hypochlorous Acid (HOCL), Hypochlorite Ion (OCL^-), Hydrochloric Acid (HCL) and Oxygen (O) that inhibit disinfecting properties.

Gaseous Chlorine

Gaseous Chlorine (CL_2) is commercially available and mostly used in disinfecting mains water.

When gaseous Chlorine (CL_2) added to water (H_2O) the following hydrolysis reaction takes place:

$$Cl_2 + H_2O = H^+ + Cl^- + HOCl$$

Sodium Hypochlorite

Sodium Hypochlorite is produced adding gaseous Chlorine (CL_2) to caustic soda (NaOH). When this is done, Sodium Hypochlorite (NaOCL), water (H_2O) and salt (NaCl) are produced according to the following r*eaction:*

$$Cl_2 + 2NaOH + \rightarrow NaOCl + NaCl + H_2O$$

Chlorine reacts with sodium hydroxide to Sodium Hypochlorite (NaOCl). Sodium Hypochlorite is known as Bleach. Bleach (NaOCL) cannot be combined with acids. When NaOCL comes in contact with acids the hypochlorite becomes instable, causing poisonous gaseous Chlorine (CL_2) to escape.

Hypochlorous Acid and Hypochlorite Ion formation

Hypochlorous Acid (HOCL) and Hypochlorite Ion (OCL^-) are the by-products of Sodium Hypochlorite (NaOCL) in water (H_2O)

NaOCL reacts with water (H_2O) to Hypochlorous Acid (HOCl) and Hypochlorite Ions (OCl^-).

$$NaOCl + H_2O \rightarrow HOCl + NaOH-$$

Hypochlorous Acid formation

Hypochlorous Acid (HOCL) is the by-product of gaseous Chlorine (CL_2) in Water. Gaseous Chlorine (CL_2) reacts with water to Hypochlorous Acid (HOCL).

$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$

Oxygen formation

Depending on the pH value, Hypochlorous Acid (HOCL) expires to Hypochlorite Ions (OCL^-).

$Cl_2 + 2H_2O \rightarrow HOCl + H3O + Cl^- \; HOCl + H_2O \rightarrow H_3O^+ + OCl^-$

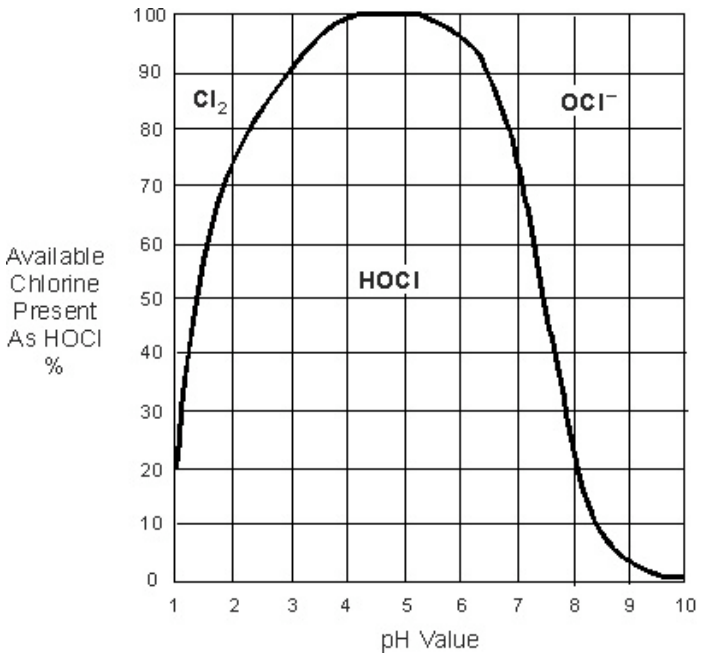
This falls apart to Chlorine and Oxygen atoms:

$OCl^- \rightarrow Cl^- + O$

The efficacy of disinfection is determined by the pH.

Disinfection will take place optimally when the pH is between 5 and 7, as then a maximum of HOCL is present.

HOCL reacts faster than OCl^- ; HOCL is 80-100% more effective than OCL^-. HOCL does not evaporate and does not cause severe corrosion like CL_2. CL_2 exposed in air can be very explosive and evaporation should be avoided. For this reason, the ideal pH is between 6 and 7, as no CL_2 is present.

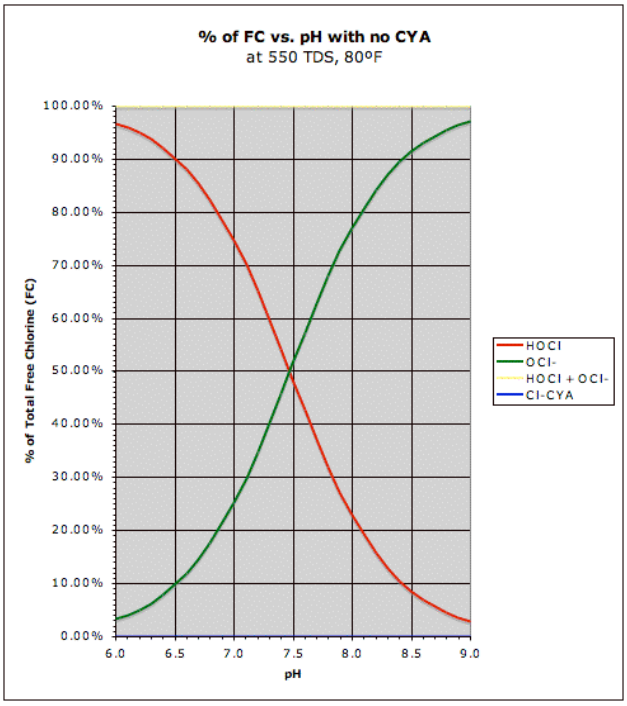


The level of HOCL will decrease when the pH value is higher than 5. The level of HOCL will decrease when the pH value is lower than 5. With a pH value of 6.5 the level of HOCL is more than 90%, whereas the concentration of OCL^- is less than 10%.

Free Available Chlorine

Free Available Chlorine (FAC) is chlorine that is present in the form of Hypochlorous Acid, hypochlorite ions or as dissolved elemental chlorine. FAC includes all chlorine species that are not combined with ammonia (or other nitrogenous compounds) to form chloramines. It is 'free' in the sense that it has not yet reacted with anything, and 'available' in the sense that it can and will react if needed.

A pH value of 6 to 7 is the most effective and the safest pH-range, due to absence of chlorine gas. Therefore when Free Available Chlorine is mentioned, it is assumed that Free Available Chlorine solely consists of HOCL and OCL^-



Free Available Chlorine compounds with regard to pH .Hypochlorous Acid (red) and Hypochlorite Ion (green)

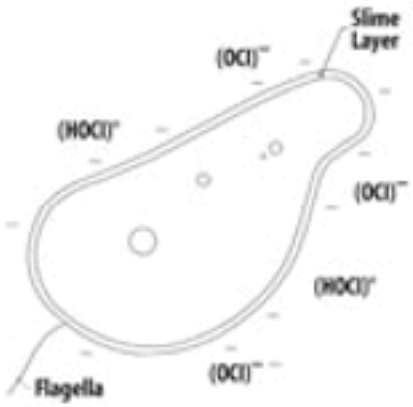
Superiority of Hypochlorous Acid compared to Hypochlorite Ion

Hypochlorous Acid (HOCl, which is electrically neutral) and Hypochlorite Ions (OCl^-, electrically negative) will form Free Available Chlorine (FAC) when bound together. This results in disinfection. Both substances have very distinctive behavior.

The cell wall of pathogenic microorganisms is negatively charged by nature. As such,

the negatively charged Hypochlorite Ion (OCL-) can only penetrate it by the neutral Hypochlorous Acid (HOCL), rather than.

HOCL can penetrate slime layers, cell walls and protective layers of microorganisms and effectively kills pathogens as a result. The microorganisms will either die or suffer from reproductive failures.



The pH neutral Hypochlorous Acid (HOCL) *can penetrate cell walls of pathogenic microorganisms whereas the negatively charged Hypochlorite Ion* (OCL^-) *cannot penetrate cell walls.*

Besides the neutrality of HOCL, it is a much more reactive and is a much stronger disinfectant than OCL^-, as HOCL is split into hydrochloric acid (HCl) and atom air Oxygen (O). Oxygen is a very powerful disinfectant.

HOCL guarantees optimal disinfecting

The disinfecting properties of Chlorine in water are based on the formation and oxidizing power of Oxygen and HOCL. These conditions occur when the pH is between 6 and 7.

HOCL produced onsite from AQUAOX ECS-200 and ECS-400 Systems has a pH of 6.5. At this pH more than 90% of the free available chlorine is HOCL, less than 10% OCL^- and no CL_2 are formed.

The strength of Free Available Chlorine (FAC) in HOCL is pre-set to 200ppm. To make a solution with 200ppm FAC from commercially available bleach (NaOCL), it is diluted in water (H_2O).

<u>The problem with diluting bleach in water is twofold:</u>

1) The volume to dilute bleach is very small. Small differences in the volume of bleach added to water causes significant differences in terms of pH and Free Available Chlorine (FAC).

2) The fact that water has naturally different pH levels, causes that addition of the same volume of bleach still result in a different pH. Although at each dilution 200ppm FAC can be measured, the pH of the mixture and consequently the amount of active compounds HOCL and OCL^- may vary considerably.

Therefore, disinfecting properties using bleach vary whereas the disinfecting properties of HOCL are kept stable. As a result HOCL may exceed the disinfecting properties of bleach by **300** times.

Safety

When producing HOCL by acidifying NaOCL, relatively high prices and possibility of side reactions limit the use of weak organic acids; use of cheaper inorganic acids provokes gaseous chlorine discharge and a raise of toxicity level. Because of it, the method above is only used for water treatment, where residual chlorine concentration values do not exceed 0.5-5mg/l.

Dilution of gaseous chlorine in water to produce HOCL according to equation demands special safety measures and is only used for disinfecting large volumes of water, where active chlorine concentration is below 10-15mg/l. Nowadays all the companies that manufacture gaseous chlorine stopped gaseous chlorine production and started NaOCL manufacture exclusively because of safety considerations.

HOCL onsite produced by AQUAOX Systems is a unique method of non-reagent synthesis of HOCL. We would like to point out once more that the unique quality of the AQUAOX System is the possibility of directed pH regulation in the 6.0-7.0 ranges, while working with solutions of any mineralization, whereas electrolyses of sodium chloride solutions have identical biocidal activity if pH and FAC concentration are equal.

Appendix 5 HACCP Plans

The following HACCP plans were obtained from the FSIS inspected facilities at Texas Tech University.

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PRODUCT DESCRIPTION

PRODUCT: BEEF SLAUGHTER

COMMON NAME:	BEEF CARCASSES, BEEF HEADS, BEEF VARIETY MEATS
HOW USED OR CONSUMED:	CARCASS, WHOLE CARCASS FABRICATION BEEF HEADS AND VARIETY MEATS FOR RETAIL SALE
TYPE OF PACKAGE:	CARCASS- NONE BEEF HEADS AND VARIETY MEATS- VACUUM PACKED
WHERE SOLD:	GENERAL PUBLIC (RETAIL SALE OR WHOLESALE DISTRIBUTION)
LABELING INSTRUCTIONS:	KEEP REFRIGERATED KEEP FROZEN SAFE HANDLING LABEL
SPECIAL DISTRIBUTION CONTROL:	KEEP REFRIGERATED

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PRODUCTS/INGREDIENTS USED TO PRODUCE PRODUCT:

MEAT/POULTRY AND BYPRODUCTS	NONMEAT FOOD INGREDIENTS	BINDERS/EXTENDERS
Live Cattle		
SPICES/FLAVORINGS	**RESTRICTED INGREDIENTS**	**PRESERVATIVES/ ACIDIFIERS**
OTHER		
Potable Water Organic Acid Spray Poly Bags String		

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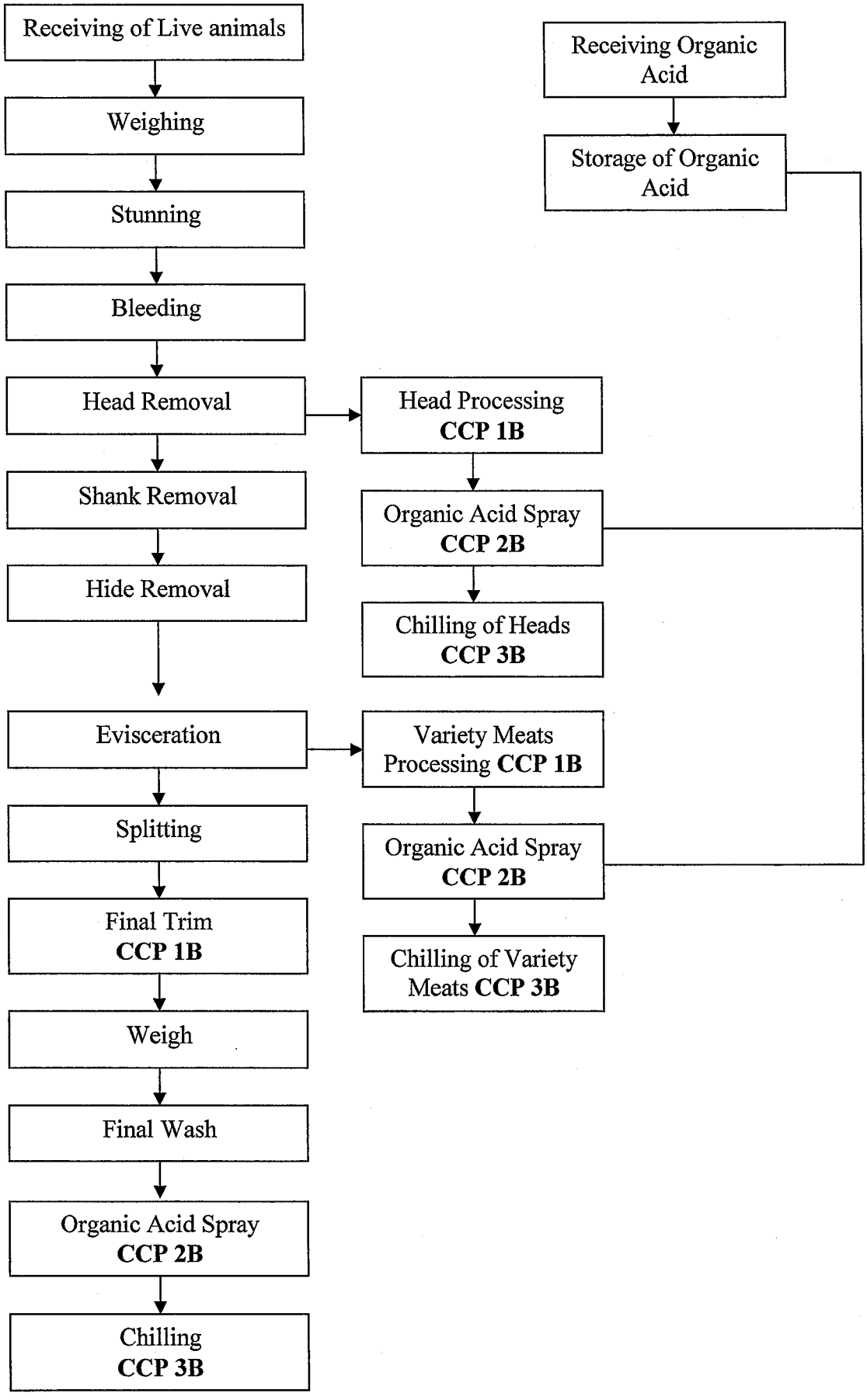
BEEF SLAUGHTER FLOW CHART



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Hazard Analysis and CCP Slaughter - Beef Slaughter

Process Step	Potential hazard (chemical, physical, biological)	Likely to occur	Justification for decision	If likely to occur, what measures could be applied to prevent, eliminate, or reduce	Is this step a CCP?
Receiving of Live Animals	**Chemical** - Residues Pesticides, and Growth Promotants	No	Unlikely to occur. USDA Residue Monitoring Program indicates that the great majority of livestock are free of violative residues when slaughtered in inspected facilities. Any animal used for research involving chemical exposure will follow protocol for proper use.		
	Physical - Buckshot Needles	No	Unlikely to occur. No reported incidences have been reported since Jan. 1, 2003.		
	Biological - Pathogens Enteric pathogens (i.e. Salmonella, E. coli O157:H7)	Yes	Live animals are potential reservoirs of pathogens. (Elder, et. al (2000) and Smith, et al. (2001))	Subsequent steps: organic acid and proper chilling of the carcass will reduce potential for pathogen growth.	No
	SRM'S/BSE	No	Pre-requisite programs address the proper removal and disposition of SRMs		
Weighing	**Chemical** - None **Physical** - None **Biological** - None				
Stunning	**Chemical** - None				
	Physical - Bone Fragments	No	Unlikely to occur. Inspection requires that the brain cavity is flushed with water.		
	Biological - None				
Bleeding	**Chemical** - None **Physical** - None				
	Biological - Pathogens Enteric pathogens (i.e. Salmonella, E. coli O157:H7 O157:H7)	Yes	Live animals are potential reservoirs of pathogens. Hide opening and sticking may introduce pathogens.	Subsequent steps: organic acid and proper chilling of the carcass will reduce potential for pathogen growth.	No

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Hazard Analysis and CCP - Beef Slaughter

Process Step	Potential hazard (chemical, physical, biological)	Likely to occur	Justification for decision	If likely to occur, what measures could be applied to prevent, eliminate, or reduce	Is this step a CCP?
Head Removal	**Chemical** - None				
	Physical - String	No	String is used to tie the esophagus & the weasand is removed during evisceration.		
	Biological - Pathogens Enteric pathogens (i.e. Salmonella, E. coli O157:H7)	Yes	Hides are potential reservoirs of pathogens. Head removal may introduce pathogens.	Subsequent steps: organic acid and proper chilling of the carcass will reduce potential for pathogen growth.	No
	BSE	No	Pre-requisite programs address the proper removal and disposition of SRMs		
Shank Removal	**Chemical** - None				
	Physical - None				
	Biological -Pathogens Enteric pathogens (i.e. Salmonella, E. coli O157:H7)	Yes	Hides are potential reservoirs of pathogens. Hide opening and removal of foreshank may introduce pathogens.	Subsequent steps: organic acid and proper chilling of the carcass will reduce potential for pathogen growth.	No
Hide Removal	**Chemical** - None				
	Physical - String	No	String is used to tie the bung. The bung is removed during evisceration.		
	Biological - Pathogens Enteric pathogens (i.e. Salmonella, E. coli O157:H7)	Yes	Hides are potential reservoirs of pathogens. Hide opening and removal may introduce pathogens.	Subsequent steps: organic acid and proper chilling of the carcass will reduce potential for pathogen growth.	No
Evisceration	**Chemical** - Organic acid	No	Unlikely to occur. A food grade organic acid is used in a recognized application method.		
	Physical - String	No	String on weasand and bung is removed.		No
	Biological - Pathogens Enteric pathogens (i.e. Salmonella, E. coli O157:H7)	Yes	Visceral contents may contain pathogens. Pathogens may be introduced during evisceration.	Subsequent steps: organic acid and proper chilling of the carcass will reduce potential for pathogen growth.	No
	BSE	No	Pre-requisite programs address the proper removal and disposition of SRMs		

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Hazard Analysis and CCP - Beef Slaughter

Process Step	Potential hazard (chemical, physical, biological)	Likely to occur	Justification for decision	If likely to occur, what measures could be applied to prevent, eliminate, or reduce	Is this step a CCP?
Splitting	**Chemical** - None				
	Physical - Metal fragments from damaged saw blades	No	Unlikely to occur. SOPs address proper saw blade maintenance.		No
	Biological -BSE	No	Pre-requisite programs address the proper removal and disposition of SRMs		
Final Trim - Zero Tolerance	**Chemical** - None				
	Physical - None				
	Biological - Visible feces, milk or ingesta	Yes	Removal of visible contamination is required by a Federal Register notice from USDA/FSIS entitled "Livestock Carcass and Poultry Carcasses Contaminated With Visible Fecal Material" published November 28, 1997.	Trim all visible feces, milk or ingesta.	Yes **CCP 1B**
Weighing	**Chemical** - None				
	Physical - None				
	Biological - None				
Final Wash	**Chemical** - None				
	Physical - None				
	Biological - None				

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Hazard Analysis and CCP - Beef Slaughter

Process Step	Potential hazard (chemical, physical, biological)	Likely to occur	Justification for decision	If likely to occur, what measures could be applied to prevent, eliminate, or reduce	Is this step a CCP?
Organic Acid Spray of Carcasses	**Chemical** - Organic Acid	No	Unlikely to occur. A food grade organic acid is used in a recognized application method.		
	Physical - None				
	Biological - Pathogens Enteric pathogens (i.e. Salmonella, E. coli O157:H7)	Yes	The proper application of organic acid can reduce pathogens.	Organic acid spray	Yes **CCP 2B**
Chilling	**Chemical** - None				
	Physical - None				
	Biological - Pathogens Enteric pathogens (i.e. Salmonella, E. coli O157:H7)	Yes	Proper chilling can reduce pathogen growth.	Temperature	Yes **CCP 3B**
Head processing	**Chemical** - None				
	Physical - Bone Fragments	No	Unlikely to occur. Inspection requires that the brain cavity is flushed with water. Brains are not sold for human consumption.		
	Biological - Pathogens Enteric pathogens (i.e. Salmonella, E. coli O157:H7)	Yes	Removal of visible contamination is required by a Federal Register notice from USDA/FSIS entitled "Livestock Carcass and Poultry Carcasses Contaminated With Visible Fecal Material" published November 28, 1997.	Trim all visible feces, milk or ingesta.	Yes **CCP 1B**
Organic Acid Spray of Heads	**Chemical** - Organic acid	No	Unlikely to occur. A food grade organic acid is used in a recognized application method.		
	Physical - None				
	Biological - Pathogens Enteric pathogens (i.e. Salmonella, E. coli O157:H7)	Yes	The proper application of organic acid can reduce pathogens.	Organic acid spray.	Yes **CCP 2B**

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Hazard Analysis and CCP - Beef Slaughter

Process Step	Potential hazard (chemical, physical, biological)	Likely to occur	Justification for decision	If likely to occur, what measures could be applied to prevent, eliminate, or reduce	Is this step a CCP?
Chilling of heads	**Chemical** - None **Physical** - None **Biological** - Pathogens Enteric pathogens (i.e. Salmonella, E. coli O157:H7)	Yes	Proper chilling can reduce pathogen growth.	Temperature	Yes **CCP 3B**
Variety Meat Processing	**Chemical** - None **Physical** - None **Biological** - Pathogens Enteric pathogens (i.e. Salmonella, E. coli O157:H7)	Yes	Removal of visible contamination is required by a Federal Register notice from USDA/FSIS entitled "Livestock Carcass and Poultry Carcasses Contaminated With Visible Fecal Material" published November 28, 1997.	Trim all visible feces, milk or ingesta.	Yes **CCP 1B**
Organic Acid Spray of Variety Meats	**Chemical** - Organic acid **Physical** - None	No	Unlikely to occur. A food grade organic acid is used in a recognized application method.		
	Biological - Pathogens Enteric pathogens (i.e. Salmonella, E. coli O157:H7)	Yes	The proper application of organic acid can reduce pathogens.	Organic acid spray	Yes **CCP 2B**
Chilling of Va Meats	**Chemical** - None **Physical** - None **Biological** - Pathogens Enteric pathogens (i.e. Salmonella, E. coli O157:H7)	Yes	Proper chilling can reduce pathogen growth.	Temperature	Yes **CCP 3B**

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Hazard Analysis and CCP - Beef Slaughter

Process Step	Potential hazard (chemical, physical, biological)	Likely to occur	Justification for decision	If likely to occur, what measures could be applied to prevent, eliminate, or reduce	Is this step a CCP?
Receiving of Organic Acid	**Chemical** - None **Physical** - None **Biological** - None				
Storage of Organic Acid	**Chemical** - None **Physical** - None **Biological** - None				

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HACCP DECISION MAKING DOCUMENTATION
Beef Slaughter

CCP 1B: ZERO TOLERANCE

Critical Limit: No visible feces, milk, or ingesta
What: Carcass, head, and variety meats
How: Visual observation
Frequency: Every carcass, head, and variety meats
Who: Carcass trimmer

USDA's Food Safety and Inspection Service requires the trimming of all visible feces, milk, and ingesta from the carcass prior to washing as part of zero tolerance control. According to 62 Federal Register (FR) 63254, November 28, 1997; the plant must address zero tolerance in the HACCP plan. Therefore, this step is identified as a CCP to control visible feces, milk, or ingesta by knife trimming any visible contamination. Currently there is no other way to identify feces, milk, or ingesta other than visually. Therefore, we have decided to use visual observation to identify FMI. Due to the low volume of animals slaughtered, we decided to visually inspect every carcass, head, and variety meat for visible FMI contamination.

VERIFCATION
Visual observation of:

1. **Designee conducting zero tolerance monitoring on one carcass, head, and variety meat per kill, or**
2. **Record Keeping**

Plant manager or designee will perform observations one time during the day of slaughter.

USDA's Food Safety and Inspection Service 9 CFR 417.4(a)(2) requires establishments to perform ongoing verification activities which include:

1. The calibration of process-monitoring instruments;
2. Direct observation of monitoring activities, and corrective actions; and
3. The review of records generated and maintained in accordance with 9 CFR 417.5(a)(3).

Due to the low volume of animals slaughtered, limited employees, and past records, we found that one carcass, head, and variety meat per kill was sufficient enough, to observe one time during the day of slaughter, for verification.

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CCP 2B: ORGANIC ACID SPRAY

Critical Limit: The concentration of organic acid solution must be at least 2%.
What: Organic acid concentration
How: Titration test kit
Frequency: Every batch
Who: Organic acid formulator

Critical Limit: Each carcass, head, and variety meat must be sprayed.
What: Application to carcass, head, and variety meat
How: Visual observation
Frequency: Every carcass, head, and variety meats
Who: Organic acid sprayer

Lactic acid has been shown to be an effective organic acid at reducing pathogen loads. FSIS Notice 49-94 (12-21-94) states that up to 2.5% of a food grade acid can be used. Therefore, we consider the use of lactic acid as a CCP for reducing pathogens. Scientific literature is also available to support the use of lactic acid as a microbial intervention that will reduce E. coli 0157:H7 and Salmonella. This intervention has been scientifically validated to reduce levels of E. coli 0157:H7 that are higher than would be anticipated on carcasses during normal processing. The titration test kit is a general laboratory technique to determine the acid concentration in a solution. Due to the low volume of animals slaughtered and the time between slaughters, we decided to visually apply an organic acid solution to every carcass, head, and variety meat.

VERIFICATION

Visual observation of:

1. **Titration kit use, and**
2. **Designee applying the organic acid spray, and**
3. **Record Keeping**

Plant manager or designee will perform observations one time during the day of slaughter.

USDA's Food Safety and Inspection Service 9 CFR 417.4(a)(2) requires establishments to perform ongoing verification activities which include:

1. The calibration of process-monitoring instruments;
2. Direct observation of monitoring activities, and corrective actions; and
3. The review of records generated and maintained in accordance with 9 CFR 417.5(a)(3).

The titration test kit is a validated procedure to verify the acid concentration of a solution, if performed properly. Therefore, we decided to observe the employee performing the titration test kit for verification. Moreover, the proper application of the organic acid spray is critical to reduce the level of enteric pathogens. Therefore, we decided to observe the application of organic acid spray one time during the day of slaughter based on the low volume of animals slaughtered, limited employees, and past records.

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CCP 3B: CHILLING

Critical Limit: ≤ 45 F sub-surface temperature 24 hours after harvest.
What: Sub-surface temperature of carcass (round), head, and variety meats
How: Calibrated thermometer
Frequency: 25% of the kill, minimum of 1 carcass, head, and variety meat per kill
Who: Manager or designee

The process of chilling does not introduce biological hazards. Pathogens that are present when entering the chilling step cannot be eliminated either. Although microbial interventions are in place to reduce pathogens on the slaughter floor, pathogens may be present on the carcass as it passes from the slaughter floor to the cooler. Proper chilling of the carcass can help reduce the potential for pathogen growth. Therefore, this step is identified as a CCP. Scientific literature supports that pathogen growth is reduced at temperatures 45 F or below. We found that the sub-surface temperature of a carcass, head, or variety meat best determines the overall temperature. Due to the low volume of animals, we determined it was sufficient enough to monitor 25 % of the kill, with a minimum of 1 carcass, head, and variety meat per kill.

VERIFICATION

Visual observation of:

1. **Designee taking sub-surface temperature of carcass, head, and variety meat, and**
2. **Record Keeping.**

Plant manager or designee will perform observations on one carcass, head, and variety meat after slaughter to insure the carcass, head, and variety meats contain no visible feces, milk, or ingesta.

The plant manager or designee will calibrate temperature-recording device weekly.

USDA's Food Safety and Inspection Service 9 CFR 417.4(a)(2) requires establishments to perform ongoing verification activities which include:

1. The calibration of process-monitoring instruments;
2. Direct observation of monitoring activities, and corrective actions; and
3. The review of records generated and maintained in accordance with 9 CFR 417.5(a)(3).

Due to the low volume of animals slaughtered, and the importance of accurate temperature measurements, we decided to verify the designee taking internal temperatures on one carcass, head, and variety meat. Furthermore, we found that weekly calibration of temperature-recording devices were sufficient enough, due to the low volume of production performed.

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Beef Slaughter Verification and Recordkeeping

PROCESS STEP/CCP	VERIFICATION	RECORDS
Trim – Zero Tolerance CCP 1B	-Visual observation of: (1) Designee conducting zero tolerance monitoring on one carcass, head, and variety meat per kill, and (2) Record Keeping -Plant manager or designee will perform observations one time during the day of slaughter.	Beef Slaughter Log Deviation/Corrective Action Log
Organic Acid Spray CCP 2B	-Visual observation of: (1) Titration test kit use, and (2) Designee applying the organic acid spray, and (3) Record Keeping -Plant manager or designee will perform observations one time during the day of slaughter.	Beef Slaughter Log Deviation/Corrective Action Log
Chilling CCP 3B	-Visual observation of: (1) Designee taking sub-surface temperature of carcass, head, and variety meat, and (2) Record Keeping -Plant manager or designee will perform observation on one carcass, head, and variety meat after slaughter to insure the carcass, head, variety meats contain no visible feces, milk, or ingesta. -The plant manager or designee will calibrate temperature-recording device weekly.	Beef Slaughter Log Deviation/Corrective Action Log Temperature Recording Device Calibration Log

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Beef Slaughter Log **Lot #** **Processing Date:**_____

Slaughter Number	Critical Limit CCP 1	SRM Removed?	Performed By	Time/Date	Critical Limit CCP 2	Performed By	Time/Date
1 C		Yes No					
1 H		Yes No					
1 V		Yes No					
2 C		Yes No					
2 H		Yes No					
2 V		Yes No					
3 C		Yes No					
3 H		Yes No					
3 V		Yes No					
4 C		Yes No					
4 H		Yes No					
4 V		Yes No					
5 C		Yes No					
5 H		Yes No					
5 V		Yes No					

CCP 1: Trim - Zero Tolerance;
Critical Limit: No visible feces, milk, or ingesta
CCP 2: Organic Acid Spray;
Critical Limit: Spray all carcasses, heads, & variety meats with at least a 2% organic acid solution

Acceptible Statements:
No Visible FMI;
Carcass Sprayed;
Head Sprayed;
VM Sprayed

	Was Monitoring Performed Correctly?		Initial	Time/Date	Was Organic Acid Applied Correctly?		Initial	Time/Date
Carcass	Yes	No			Yes	No		
Head:	Yes	No			Yes	No		
VM:	Yes	No			Yes	No		
Record Keeping	Yes	No			Yes	No		

CCP Verification

CCP 2: Organic Acid Concentration 10 drops = 1% solution and 20 drops = 2% solution

Amount of Acid	Amount of Water	Number of Drops/ %	Performed By	Time/Date

CCP Verification:			Initial	Time/Date
Was Titration Kit Used Correctly?	Yes	No		
Record Keeping Performed?	Yes	No		

Pre-shipment
Review Signature:_____

Approved Date:_____ Time:_____

CCP 3: Chilling
Critical Limit: ≤ 45 F Sub-Surface 24 hours post harvest

Carcass #	Temp. F	Intial	Time/Date
Head			
VM			

CCP Verification:	Monitoring Performed Correctly?		Record Keeping Performed Correctly?		Intial	Time/Date
Carcass	Yes	No	Yes	No		
Head	Yes	No	Yes	No		
VM	Yes	No	Yes	No		

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Deviation/Corrective Action Log

1. Identify and eliminate cause of deviation.
2. Bring CCP under control after corrective action is taken.
3. Measures to prevent recurrence are established.
4. No product that is injurious to health or adultered enters commerce.
   All requirements of 9 CFR 417.3 will be met by manager or designee.

CCP No.	Product I.D.	Deviation	Corrective Action No. 1	Corrective Action No. 2	Corrective Action No. 3	Corrective Action No. 4	Performed By	Time

Pre-shipment Review Signature:_____ Approved Date:_____ Time:_____

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Weekly Temperature Recording Device Calibration Log

Date	Time	Temperture Recording Device Identification	Temperature Reading at: 32 F	212 F	Validating Thermometer Reading	Action Taken	Performed By

Date	Time	Temperture Recording Device Identification	Temperature Reading at: 32 F	212 F	Validating Thermometer Reading	Action Taken	Performed By

Date	Time	Temperture Recording Device Identification	Temperature Reading at: 32 F	212 F	Validating Thermometer Reading	Action Taken	Performed By

Appendix 6 Research papers of relevance

Escherichia coli O157 Prevalence and Enumeration of Aerobic Bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at Various Steps in Commercial Beef Processing Plants

Authors: Arthur T.M.[1]; Bosilevac J.M.[1]; Nou X.[2]; Shackelford S.D.[1]; Wheeler T.L.[1]; Kent M.P.[1]; Jaroni D.[2]; Pauling B.[3]; Allen D.M.[3]; Koohmaraie M.[2]

Source: Journal of Food Protection®, Volume 67, Number 4, 1 April 2004 , pp. 658-665(8)

The effectiveness of current antimicrobial interventions used in reducing the prevalence or load of *Escherichia coli* O157 and indicator organisms on cattle hides and carcasses at two commercial beef processing plants was evaluated. Sponge sampling of beef cattle was performed at five locations from the initial entry of the animals to the slaughter floor to the exit of carcasses from the "hotbox" cooler. For each sample, *E. coli* O157 prevalence was determined and total aerobic bacteria, *Enterobacteriaceae*, and *E. coli* O157 were enumerated. *E. coli* O157 was found on 76% of animal hides coming into the plants, but no carcasses leaving the cooler were identified as contaminated with *E. coli* O157. A positive relationship was seen between the incidence of *E. coli* O157 in hide samples and that in preevisceration samples. Aerobic plate counts and *Enterobacteriaceae* counts averaged 7.8 and 6.2 log CFU/100 cm^2, respectively, on hides, and 1.4 and 0.4 log CFU/100 cm^2, respectively, on chilled carcasses. Aerobic plate counts and *Enterobacteriaceae* counts on preevisceration carcasses were significantly related to the respective levels on the corresponding hides; the carcasses of animals whose hides carried higher numbers of bacteria were more likely to carry higher numbers of bacteria. Implementation of the sampling protocol described here would allow processors to evaluate the efficacy of on-line antimicrobial interventions and allow industrywide benchmarking of hygienic practices

Decontamination of Beef Subprimal Cuts Intended for Blade Tenderization or Moisture Enhancement

Authors: Heller, C.E.[1]; Scanga, J.A.[1]; Sofos, J.N.[1]; Belk, K.E.[1]; Warren-Serna, W.[2]; Bellinger, G.R.[2]; Bacon, R.T.[3]; Rossman, M.L.[4]; Smith, G.C.[1]

Source: Journal of Food Protection®, Volume 70, Number 5, May 2007 , pp. 1174-1180(7)

The prevalence of *Escherichia coli* O157:H7 on beef subprimal cuts intended for mechanical tenderization was evaluated. This evaluation was followed by the assessment of five antimicrobial interventions at minimizing the risk of transferring *E. coli* O157:H7 to the interior of inoculated subprimal cuts during blade tenderization (BT) or moisture enhancement (ME). Prevalence of *E. coli* O157:H7 on 1,014 uninoculated beef subprimals collected from six packing facilities was 0.2%. Outside round pieces inoculated with *E. coli* O157:H7 at 10^4 CFU/100 cm^2 were treated with (i) no intervention, (ii) surface trimming, (iii) hot water (82 C), (iv) warm 2.5% lactic acid (55 C), (v) warm 5.0% lactic acid (55 C), or (vi) 2% activated lactoferrin followed by warm 5.0% lactic acid (55 C) and then submitted to BT or ME. Prevalence (n = 196) of internalized (BT and ME) *E. coli* O157:H7 was 99%. Enumeration of *E. coli* O157:H7 (n = 192) revealed mean surface reductions of 0.93 to 1.10 log CFU/100 cm^2 for all antimicrobial interventions. *E. coli* O157:H7 was detected on 3 of the 76 internal BT samples and 73 of the 76 internal ME samples. Internal ME samples with no intervention had significantly higher mean *E. coli* O157:H7 populations than did those internal samples treated with an intervention, but there were no significant differences in *E. coli* O157:H7 populations among internal BT samples. Results of this study demonstrate that the incidence of *E. coli* O157:H7 on the surface of beef subprimal cuts is low and that interventions applied before mechanical tenderization can effectively reduce the transfer of low concentrations of *E. coli* O157:H7 to the interior of beef subprimal cuts.

Validation of a Lactic Acid- and Citric Acid-Based Antimicrobial Product for the Reduction of *Escherichia coli* O157:H7 and *Salmonella* on Beef Tips and Whole Chicken Carcasses

Authors: Laury, A.M.[1]; Alvarado, M.V.[1]; Nace, G.[2]; Alvarado, C.Z.[1]; Brooks, J.C.[1]; Echeverry, A.[1]; Brashears, M.M.[1]

Source: Journal of Food Protection®, Volume 72, Number 10, October 2009 , pp. 2208-2211(4)

The objectives of this study were to determine the effects of a lactic acid- and citric acid-based antimicrobial product on the reduction of *Salmonella* on whole broiler carcasses during processing and the reduction of *Salmonella* and *Escherichia coli* O157:H7 on beef trim. Freshly harvested broiler carcasses were inoculated with an inoculum of *Salmonella* strains to yield a 10^5 CFU/ml pathogen load on the surface of the carcass. The beef tips were inoculated as well with an inoculum of either *E. coli* O157:H7 or *Salmonella* to yield 10^4 CFU/100 cm^2. After 30 min for attachment, the broiler carcasses were treated with Chicxide applied for 5 s via a spray or immersed in Chicxide for 5, 10, or 20 s. Broiler carcasses were rinsed in poultry rinse bags with 400 ml of Butterfield's phosphate buffer in which *Salmonella* was enumerated from the diluents and Butterfield's phosphate. Chicxide significantly reduced *Salmonella* by 1.3 log CFU/ml with spray treatment and 2.3 log CFU/ml for all dip treatments. Following 30 min of attachment, the beef tips were placed into a spray cabinet with either Beefxide or sterilized water (control) and sprayed at 1 ft/2.5 s chain speed at 40 lb/in^2. The external surface of each beef tip was swabbed (100 cm^2) to determine pathogen loads. Beefxide significantly reduced *E. coli* O157:H7 by 1.4 log CFU/100 cm^2 and *Salmonella* by 1.1 log CFU/100 cm^2 ($P < 0.05$) compared with the control samples.

Appendix 7 Validation of antimicrobial interventions for small and very small processors: a how-to guide to develop and conduct validations

PEER-REVIEWED ARTICLE

Food Protection Trends, Vol 33, No. 2, p. 95–104

6200 Aurora Ave., Suite 200W, Des Moines, IA 50322-2864

Validation of Antimicrobial Interventions for Small and Very Small Processors: A How-to Guide to Develop and Conduct Validations

CONSORTIUM OF FOOD PROCESS VALIDATION EXPERTS (CFPVE)[1]*

[1] The CFPVE consists of representatives from Auburn University, Colorado State University, Iowa State University, Kansas State University, Oklahoma State University, Pennsylvania State University, Texas A&M University, Texas Tech University, the United States Department of Agriculture — Agricultural Research Service, the University of Arkansas, the University of Nebraska, and the University of Wisconsin.

SUMMARY

It is important to assure that antimicrobial interventions applied on/into foods to control pathogenic microorganisms are functioning properly and achieving the desired goal of preventing, reducing and/or eliminating microbial hazards associated with a defined food product. This approach is necessary both to ensure that antimicrobial interventions are having the desired positive effect on food safety and to provide assurance to the processor that the investment in food safety is in fact providing the appropriate benefit for the investment. Validation is a fundamental component of the HACCP system, in that those processors currently required to have HACCP plans in place are also required to validate their HACCP plans. This manuscript provides a practical approach for developing validation protocols to evaluate the efficacy of antimicrobial interventions, especially for small and very small processors.

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INTRODUCTION

Assuring safety of food from production to consumption is a complicated process requiring an organized, deliberate approach to preventing and controling potential food safety hazards. The Hazard Analysis and Critical Control Point (HACCP) system is widely accepted as the most effective and logical approach to accomplishing this task. HACCP plans are developed on the basis of seven principles: hazard analysis, identification of CCPs, establishment of critical limits, monitoring of CCPs, defining of corrective actions, verification, and record-keeping/documentation. Of these seven principles, verification procedures may be the most misunderstood and least effectively implemented, and are often overlooked or given low priority.

The National Advisory Committee on Microbiological Criteria for Food (NACMCF) *(5)* defines verification as any activity, other than monitoring, that determines the validity of the HACCP plan and ensures that the HACCP system is operating according to the plan. Included in verification activities is validation, defined by NACMCF as the element of verification focused on collecting and evaluating scientific and technical information to determine whether the HACCP plan, when properly implemented, will effectively control the defined hazards.

Before a HACCP plan can function with assured control, it must be determined that all hazards have been identified and that specific control measures are scientifically sound and will be effective when implemented. Validation, both of individual CCPs and the entire HACCP plan, is integral to determining the plan's soundness. A HACCP plan that has not been validated may appear logical and effective; however, without thorough validation of the process, there is no assurance that factors that may compromise product safety have been evaluated. Process control and safety cannot be assured unless a HACCP plan has been validated.

The Food and Drug Administration (FDA), through implementation of the Food Safety Modernization Act (FSMA), and the United States Department of Agriculture's Food Safety and Inspection Service (FSIS) will require all food processors to provide evidence of HACCP plan validation. Proper validation of HACCP controls may be difficult to implement for all food processing operations; however, small to very small processors may find the task to be particularly burdensome. This manuscript provides a practical overview of validation, including experimental design, implementation and application, to help small, local, artisan and very small food processors to understand the concepts and protocols for validation of CCPs and HACCP plans. Basic concepts presented herein are applicable to all food processors; however, the primary focus is directed toward small to very small processors, including local and artisan manufacturers. This manuscript also discusses the importance of validation, as well as the selection of scientific justification documents to support intended process control measures. In addition, a practical approach to in-plant validation is provided, including appropriate microbiological testing, analysis and reporting.

The USDA Food Safety and Inspection Service regulations *(7)* state that "upon completion of the hazard analysis and development of the HACCP plan, the establishment shall conduct activities designed to determine that the HACCP plan is functioning as intended." Further, the Pathogen Reduction HACCP Rule states that "During this HACCP plan validation period, the establishment shall repeatedly test the adequacy of the CCPs, critical limits, monitoring and recordkeeping procedures, and corrective actions set forth in the HACCP plan (emphasis added)." In addition to other validation activities, review of the processing records themselves, routinely produced by monitoring of the HACCP system in the context of other validation activities, is a key element of HACCP plan validation.

Thus, the validation process has two aspects: (1) verifying that the antimicrobial intervention (such as a lethality process) will achieve its intended purpose of preventing, reducing and/or eliminating the hazard as implemented in the food processing operation and (2) verifying that the critical limits of the critical parameters that would impact the efficacy of the antimicrobial treatment are being met on a continual basis as implemented in the processing operation. The first aspect can be achieved only by evaluating the prevalence and/or concentrations of the organism of concern (food safety hazard). The second aspect can be achieved through review of records and by assuring that critical limits of the critical parameters are being met for the particular antimicrobial intervention in practice. These two aspects are essential components of the validation process, and assuring compliance with one aspect without the other will not assure that the antimicrobial intervention is achieving its intended purpose of preventing, reducing and/or eliminating the hazard.

Scientific and technical justification

Initial validation of the HACCP plan can be based upon various types of information, but most often utilizes scientific studies and advice of experts, regulatory guidance, industry standards or guidance, modeling programs, and university extension publications, as well as observations and data collected in the processing facility.

The most common approach to validating a process or demonstrating process control in plants is to use scientific publications that provide information on efficacy of control measures. Typically, scientific information can consist of peer-reviewed journal articles, a documented scientific study, in-house data, or data generated from published guidelines. The five primary types of scientific supporting documentation (see http://www.fsis.usda.gov/Science/HACCP_Validation/index.asp#2) are:

1. published processing guidelines (safe harbors) that achieve a stated reduction of a pathogen, such as the time-temperature guidelines in Appendix A of the final rule "Performance Standards for the Production of Certain Meat and Poultry Products";
2. a scientific article published in a peer-reviewed journal that describes the process and level of reduction of a particular food safety hazard or process stabilization; the publication or scientific article being used, however, should closely relate to the manufacturing process being validated (meet the critical parameters) with respect to species, product characteristics, processing parameters, and equipment;
3. a microbial challenge study or inoculated pack study (with non-pathogenic surrogates or indicator organisms as acceptable alternatives to the food safety hazard [pathogen]

of interest) that is designed to determine lethality or stabilization of a process; these studies are typically performed in a laboratory or pilot plant by a processing authority or expert, and it is not advocated that pathogens be introduced into the food processing plant environment;

4. data gathered in-house, which can be used to validate an antimicrobial intervention or process and which may be generated if the establishment has not implemented a process documented in the literature, and
5. regulatory performance standards as defined in the Code of Federal Regulations that outline specific prescribed procedures such as time/temperature combinations, product storage conditions, or product reconditioning procedures.

Several resources for scientific publications or pre-existing supporting documents can be found on the Internet or via county Extension agents, industry trade groups, and university libraries. Although there are several sources/access points at which information can be obtained, it is important to identify and utilize scientific information that is truly relevant to the process or the product being evaluated. In selecting scientific publications or articles relevant to the process, it is critical to look for processing parameters consistent with the specific plant's operational parameters for the product and pathogen(s) of interest. In addition to collecting pre-existing scientific and technical information, plants should obtain necessary data by repeatedly testing the adequacy of the process in preventing, reducing and/or eliminating the identified hazard and establish that the HACCP system meets the designed parameters to achieve the intended results.

In addition, the basic composition of the food, as well as the processing methods and storage conditions, should be considered in the initial analysis *(3)*. For example, processing plants often incorporate antimicrobial interventions or processes to reduce levels of certain pathogens and use published scientific support to implement that process as the first step. However, processors should demonstrate the capabilities of these new/altered interventions within specific plant environments to verify that the process step actually achieves the effect documented in the scientific study. This approach is critical because laboratory conditions often differ from conditions in the establishment, as conditions are highly controlled and on a smaller scale in the laboratory than in a processing plant; hence, specific log reductions or the ease of monitoring critical parameters achieved in the laboratory may not be readily attainable in a commercial processing operation.

Practical demonstration

Validation may be accomplished by an in-plant demonstration of achieving or meeting the critical parameters that have been identified in the scientific and technical literature. The following section discusses the main components of developing an in-plant validation process to demonstrate its effectiveness in controlling a particular food safety hazard or concern. These general considerations may be applied to a variety of processes, and may not be relevant to each distinct process.

The fundamental question in developing an in-plant demonstration is "What are you trying to validate?" While the answer to this question may seem obvious, it is in fact complex and requires considerable thought before proceeding. Initially, the answer to the question may appear to be that you are trying to validate that the product is safe. However, safety is difficult to prove, and the answer to the question should specify what the process, or a specific step in the process, is intended to accomplish.

Experimental design

The scientific and technical justification for validation provides insight regarding the expected outcome of a specific process. For example, in the scientific literature, a hot water wash is reported to have a certain impact on a specific pathogen or indicator organism for a target pathogen on a defined food product. The in-plant demonstration is intended to show that under the circumstances specific to that particular processing operation, the same result can be reproducibly/repeatedly achieved. So the answer to the question of "what is being validated" should refer to the initial justification for the use of a process. If a hot water wash is being used as an intervention, then demonstrating that the hot water wash, as described in the literature, has the same effect in your operation is the answer to the question of "what." In general, the in-plant demonstration should cover the specific interventions identified in the HACCP plan as critical control points and show that the entire process improves the microbiological safety of the product by preventing, reducing, and/or eliminating the food safety hazards identified in the hazard analysis through validation of the HACCP system.

After the initial question of "what" has been answered, a specific experiment must be designed to demonstrate both the effectiveness and the control of the process. The experiment should evaluate all of the relevant parameters previously identified in the scientific and technical justification, including but not limited to temperature, time, process speed, application pressures, and any other factors critical to a given step in the process. For both the demonstration and the reporting of the results, exact parameters and their expected ranges should be recorded. Water temperature may vary during the course of the day, so recording the target temperature as well as the variation is important in evaluating a process. It is equally important for a processor to understand and document similar variations associated with all critical parameters defined in the HACCP plan, as they may impact the effectiveness of the antimicrobial interventions being utilized. If peer-reviewed scientific research articles have been used as part of the technical justification, these articles may be useful in designing and conducting a similar in-plant demonstration.

Pathogens

If the objective is to demonstrate a reduction in the prevalence and/or levels of microorganisms, either pathogens or pathogen indicators, several additional considerations are available to evaluate the process. The choices include naturally occurring microflora, which may consist of indicator organisms, which are nonpathogenic surrogates intentionally inoculated into or onto a product for validation purposes. In general, the use of pathogens for in-plant demonstration

projects is not encouraged, unless the pathogen occurs naturally and with sufficient frequency as part of the normal microflora of the product (e.g., *Campylobacter* spp. in poultry). Artificially inoculating pathogens in a processing plant setting may compromise employee safety and product safety, and it may create sanitation and/or regulatory problems. In addition, proper disposition of contaminated product is of critical importance *(6)*, and the use of pathogens for inoculum to be used in in-plant studies would make this even more difficult.

Indicators and surrogates

In some cases, the naturally occurring microflora may serve as a useful indicator of process control. However, interpreting the results of a general aerobic population count requires knowledge of the initial microflora population. For example, if the naturally occurring microflora contains a high proportion of sporeforming bacteria, a hot water wash may appear to have little or no impact on the total aerobic population, because sporeformers are quite heat resistant. On the other hand, the presence of naturally occurring coliforms or generic *Escherichia coli* may be useful in evaluating a process, assuming that the populations are high enough to measure reliably. However, if the naturally occurring population is typically present at the lower limit of the detection method, it may not be possible to demonstrate an effect of a process.

A possible solution to this problem is the use of surrogates that can be inoculated into or onto a product at sufficient populations to demonstrate the efficacy of an antimicrobial treatment. Numerous acceptable surrogates are available, but most have been tested or designed to be used with a specific process or product *(4)*. For example, *Enterococcus faecium (1)* has been shown to be a useful surrogate for the thermal processing of almonds. Other examples are given in Table 1. It is important to match the surrogate to the intended use, as a surrogate shown to be useful for one process may not be useful for another. The production of sufficient volumes of surrogate organisms for inoculation purposes requires use of a laboratory, and therefore may be beyond the capability of some processors. In this case, the services of a research or contract laboratory may be retained to produce and supervise the use of surrogates for an in-plant demonstration. The processor must confirm with the appropriate regulatory body that the surrogate(s) being used, how they are applied, and disposition of the production units involved are acceptable prior to initiating studies.

TABLE 1. Examples of indicators and surrogates that may have application in validation studies. Specific cultures may be obtained from the American Type Culture Collection (www.ATCC.org)

Indicator or Surrogate	Strengths	Weaknesses
Mesophilic aerobic bacteria (Total Plate Count)	Easy to test for Present in every sample	Unlikely to represent pathogen population
Coliforms	Easy to test for Present in many samples	May only represent enteric pathogens
***Escherichia coli* Biotype I/II ("generic" *E. coli*)**	Easy to test for May be present in some samples	May not be present in all samples, or in populations great enough to measure
***E. coli* surrogates (ATCC 1427,1428,1429,1430,1431)**	Representative of *E. coli* O157 and salmonellae in meat products Allowed by USDA-FSIS for in-plant studies	Requires microbiologist to supervise inoculation May not represent all processes
***Enterococcus faecium* (ATCC 8459)**	Representative of thermal processes, especially with almonds and tree nuts	May have limited applicability to meat and poultry Requires microbiologist to supervise inoculation
***Pediococcus* spp.**	Readily available as starter culture Easy to inoculate	May not represent pathogen
Lactic acid bacteria	Readily available as starter culture Easy to inoculate	May not represent pathogen

Design of In-plant demonstrations

Several considerations must be addressed in the design of in-plant demonstrations. One of the most important is understanding of the expected variation that may occur under normal plant operations. An in-plant demonstration study should essentially represent a "worst case" scenario. Most operators have a general idea of the type, magnitude, and periodicity of variation that may occur within the process, based on their practical experiences with good, normal and bad days. The impact of seasonal differences should also be included in understanding this variation, which is important to answering the questions of "how much data do I need" and "how many times do I need to repeat the demonstration?" There are several statistical approaches to answering questions related to the nature and number of samples to be tested; however, some general guidelines can be applied in decision making *(8)*. There is a meaningful difference between repetitions and replications. Repetitions are multiple samples taken during the same replication; they improve the accuracy of the results, by accounting for variation within the replication. Replications are completely independent from each other, differing by lots, shifts or days; the intent of multiple replications is to accurately reflect the normal variation that occurs during the process. A more thorough discussion of variation is presented in the Data Analysis section, and those who are not familiar with this topic may want to read that section before proceeding.

The demonstration should be independently replicated at least three times. In situations where more variation is expected in the results, more data will be required to demonstrate the effectiveness of the process. Data is available on the results of the intervention (the "after" treatment in a "before" and "after" comparison), a better estimate of the number of independent replications can be determined. Table 2 provides additional suggestions regarding the number of replications required based on the expected variation. To use the table, determine the variance of the data that is expected or available from prior experience. For example, if there are 5 samples, five replicates would be required to reliably detect a statistical difference of 1.0 $\log_{10}$ CFU in a comparison of the "before" and "after" samples. As a starting point, with no available data, it would be appropriate to assume a variance of 0.5. From a realistic point of view, population reductions of < 1 $\log_{10}$ CFU/g may not have practical significance.

Other details that should be considered in the design of an in-plant demonstration include determining the location of the sampling sites within the process flow, the types of samples to be collected (e.g., sponge sample, product sample, surface excision, etc.), and the methods of analysis. This includes where and how you will collect

TABLE 2. Guidelines for the number of replications required for a given number of samples and variance, based on a 95% probability of detecting a difference of 1.0 $\log_{10}$ unit in population

Number of Samples	Variance	Difference	Number of Replicates
3	0.25	1.0	4
	0.50	1.0	9
	0.75	1.0	13
5	0.25	1.0	2
	0.50	1.0	5
	0.75	1.0	7
8	0.25	1.0	2
	0.50	1.0	4
	0.75	1.0	5
10	0.25	1.0	2
	0.50	1.0	3
	0.75	1.0	5

The basis for Table 2 can be found in van Emden *(8)*. Briefly, the Least Significant Difference can be calculated using the following formula:

$$\text{LSD } t^* \sqrt{2^* (\text{variance}/2)} =$$

where LSD is the least significant difference, t is the t statistic for a 95% with n-1 degrees of freedom, and the variance is the variance of the samples. This equation becomes:

$$\text{Number of replicates} = \frac{t^2 * 2 * \text{variance}}{\text{Difference}^2}$$

where t is the t statistic for a 95% with n-1 degrees of freedom, the variance is the variance of the samples, and the difference is the least difference which may be statistically resolved under these conditions.

TABLE 3. Two data sets with equal averages but different variances

Observation	Data Set 1	Data Set 2
1	10	25
2	20	25
3	30	30
4	40	35
5	50	35
Mean[a]	30	30
Range	40	10
Variance	250	25
Standard Deviation	15.8	5

[a]See Table 4 for formulae.

TABLE 4. Mathematical formulas for statistical calculations, for the data set

Value	Formula	Example	Excel Function[a]
Average or Mean	Sum of all data point Total number of data points	$\frac{1+2+3+4+5}{5}$	**= average ($a_1 \ldots a_x$)** Where x = the last cell in the data
Range	Maximum value = minimum value	5 - 1 = 4	**= max ($a_1 \ldots a_x$) - min ($a_1 \ldots a_x$)** Where x = the last cell in the data
Variance	Sum of each data point - average, squared, divided by the total number of data points	$\frac{\sum (\text{data point - average})^2}{5}$	**= var ($a_1 \ldots a_x$)** Where x = the last cell in the data
Standard Deviation	The square root of the sum of each data point - average, squared, divided by the total number of data points - 1	$\sqrt{\frac{\sum (x\text{-average})^2}{(N-1)}}$ Where "x" is each data point, and "n" is the number of samples in the data set	**= stdev ($a_1 \ldots a_x$)** Where x = the last cell in the data

[a] Excel, Microsoft. Mention of a specific product does not constitute an endorsement or recommendation of the product by either the authors or the International Association for Food Protection.

supporting process parameter data during the in-plant demonstration (e.g., pH, temperature, spray pressures, dwell times, etc.). Practical considerations may also affect the sampling site location, related to access to the food product. If a specific process is being evaluated, samples should be collected close to the beginning of the process and immediately after the process. For a hot water wash, the samples would be collected immediately before and immediately after the wash. The samples should be collected in a manner that neither introduces new contamination into the sample nor allows for the increased destruction of bacteria. The Food and Drug Administration, in its Bacteriological Analytical Manual (BAM) *(2)*, provides instruction on the appropriate means of collecting and handling samples, and the actual method should be documented for the report. Collecting samples using an alternate method is acceptable as long as justification for the method is provided. It is advisable to discuss sampling and analysis plans with a trained microbiologist before initiating in-plant studies.

Sample analysis

The basic properties of the method of microbial analysis need to be documented prior to the beginning of the demonstration project. The minimum level of detection, and in the case of presence/absence tests, the rate of false positive and false negatives, need to be documented. The sensitivity and specificity of the method of analysis may impact the design of the demonstration, especially if the demonstration involves the use of naturally occurring microflora. If the minimum detection limit of the analytical method is close to the typical populations encountered in the food, it may not be possible to demonstrate an effect between the initial and final populations.

Either an internal (in-house) or external laboratory may conduct the study, analyze the samples and report the results. If the samples are analyzed at an internal laboratory, it is important to clearly document that the appropriate procedures have been followed in the analysis, including not only the method used, but also the details of the method. Again, both the Microbiological Laboratory Guidebook (MLG; USDA-FSIS) and BAM provide clear, detailed methods recognized by the respective regulatory agencies. As an alternative, some methods are approved by the Association of Official Analytical Chemists (AOAC) and the methods can vary as to basic principles used for detection or the sensitivity or specificity of detection. Because of differences between methods, it is important that the appropriate method be used for the food being evaluated. If the samples are analyzed internally, it is important to document the internal quality control procedures used in the laboratory to assure that the results are reliable. If an external laboratory analyzes the samples, it is important that the external laboratory have its own procedures for ensuring quality control, whether that is ISO accreditation or another program. Prior to the start of the studies, responsible individuals within the company should understand clearly how the external laboratory will receive, store, analyze and report the needed data. Responsible individuals should ask questions and make adjustments if necessary to avoid ending up with less than optimal data and additional expense. Questions such as what the laboratory does when it receives samples on Friday and will analyze them on Monday must be considered, as this could substantially impact the results of the study.

Data analysis

Once the sample analysis is completed, the results will need to be analyzed statistically. The first step in statistical analysis is to review the actual sample results (data) as they are returned from the laboratory, an important first step in identifying any sample result that does not appear to be logical. There is always the possibility that data may be recorded incorrectly, and data analysis is only as good as the raw data being analyzed. Obvious transcription errors should be corrected (for example, pH 46.3 rather than 4.63) before the statistical analysis is conducted. Other analytical data that seem out of place or are clearly outliers should be investigated to ensure that the values in question are not errors attributable to either sample collection or analysis. The sample results cannot be excluded simply because it does not fit the expected pattern, whereas it can be excluded if there is a legitimate reason, such as a known sampling error. Any data eliminated from the final analysis must be accompanied by a written justification based on known facts. One purpose of an in-plant demonstration is to learn more about the actual process as implemented in the processing operation; therefore, deliberately excluding data from the analysis for reasons that cannot be justified not only weakens the validation process but also ignores information that is valuable in understanding the process.

Data analysis involves more than simply calculating the average. Table 3 illustrates this point by presenting two sets of data with identical averages. A measure of the variability associated with the results is necessary to put the data into context. Several measures, including the variance, standard deviation and standard error, indicate the degree of variability. These values may be calculated using the formulas shown in Table 4.

Several computer software programs are available that can assist in performing basic statistical analysis. One of the most popular spreadsheet programs has several statistical functions as part of the program, including a one-way analysis of variance (ANOVA), and the on-line tutorial will guide the user through the process. Other inexpensive statistical analysis programs are available and provide adequate means for analyzing data.

The results of the data analysis will determine if there is a statistical difference between the "before" and "after" samples, as well as providing an estimate of how significant the difference is. These results allow the processor to demonstrate that under their specific plant process and environment, a certain result can reasonably be expected to occur within some confidence limits. This result must be viewed in the context of the original question that was to be evaluated, and cannot be extended beyond that specific process. The strength of this claim is only as strong as the initial design of the demonstration, the number of samples and replications, the sample analysis methods utilized, and the statistical analysis applied.

Conclusions and reporting

An in-process demonstration provides evidence for what a process is capable of accomplishing during normal operations. Validation reflects the system's performance under the conditions and parameters defined in the study. Changes to these parameters do not necessarily mean that a new demonstration must be performed. For example,

raising the water temperature by 10°F or increasing the contact time by 5 seconds probably would not require a new in-plant demonstration, as those changes would be expected to result in greater reductions of a microbial hazard or a better control of the hazard. However, major changes that might allow for increased pathogen survival, such as lowering water temperatures, reducing contact times, changing spray nozzle types or distances, reducing pressure, or changing the supplier of a previously validated antimicrobial must be shown to produce results equivalent to previously evaluated conditions. In most cases, this will require a new in-plant demonstration.

In-plant demonstrations are process- and facility-specific. While the results may be generalized to other processes used in other processing facilities, the information developed in one facility could be part of the scientific and technical justification of a demonstration performed in another facility. However, validation of a process in one facility cannot suffice as a validation of the same process in another facility. Local or regional differences in equipment, water quality, and individual processes are such that each processing facility must conduct its own in-plant demonstration of each process.

At the conclusion of an in-plant demonstration, it is important to document the demonstration for future reference. This in-plant demonstration may be incorporated into the supporting documentation for a HACCP plan or may be used as necessary to meet regulatory requirements. From a practical standpoint, in-plant demonstrations require considerable planning, time to conduct the demonstration, and sample analysis costs. It is important to record this information in a formal report to obtain the maximum return on investment.

A report should include the following information. First, the dates, time and location of the demonstration, and lead personnel involved (including expert advisers/consultants and external laboratories) should be recorded. Any approvals requested and received (e.g., use of surrogate organisms and product disposition guidance) should be documnented, so that as personnel and processes change, the information can be viewed in the context of when it was performed. All relevant information, beginning with the initial question to be evaluated, should be clearly explained. Because the report is a means of communicating to others, both within and external to the company, what was attempted and accomplished in the demonstration, all details should be included. Although some details may seem obvious and unnecessary to include, a regulatory official who has never been in the facility may review the report, and it is thus important to clearly explain how the demonstration was conducted and how the results were evaluated. The report should also include the actual sample analysis data and all of the calculations used in the analysis. If a computer program for statistical analysis is used, the name of the program as well as the specific procedures used should be described. A printout of the results should be included as part of the results. An example of a report format is shown in Table 5.

Other considerations

If a product is inoculated with a surrogate organism during the demonstration, consideration needs to be given to the disposition of the product. A raw product that will be cooked by a further processor, may present no additional concerns; however, if the product would not normally be cooked sufficiently by the consumer, it may be necessary to divert the product to an alternate process or end user where use of the product will be under more control than it would be if used by the general consumer.

Where to start

An in-plant demonstration project is important to understanding specific process capabilities as well as for meeting regulatory requirements. Although it may appear to be complicated, as with any process, it can be broken down into specific tasks, a general outline of which is shown in Table 6.

When preparing to conduct an in-plant demonstration, all necessary resources must be assembled prior to beginning the validation. Basic questions such as who will do which step in the demonstration, from collecting samples to analyzing the samples, must be addressed. Accurate written procedures for each type of sampling and appropriate training of personnel involved with the study are absolutely crucial. Having the necessary sampling materials pre-labeled is an important detail that makes the process of sample collection easier. If the samples are being sent to an external laboratory, having the necessary shipping items on hand (boxes, coolers, cold packs, shipping temperature recorders, etc.) is important prior to beginning the project. As previously mentioned, pre-study communication and agreement on expectations of both entities (processor and laboratory) is imperative.

TABLE 5. Generalized outline of a report format

1. **Initial Details:**
   - Who did the study (key personnel)?
   - When was it done?
   - Where was it done?
   - What process was evaluated?
2. **What was the question to be evaluated?**
   - What was the overall design?
   - What samples were collected?
   - How many samples were collected?
   - How were the samples collected to assure independent replication?
   - How were the samples analyzed?
   - What laboratory quality assurance programs were in place?
   - Where were the samples analyzed?
3. **What were the results?**
   - Overview of raw data
   - Overview of data analysis
   - Results of data analysis
4. **Conclusions**
5. **Other considerations**
   - Disposition of product
6. **Appendices**
   - Table of sample data
   - Table of data analysis results (may include graphs)
   - Printout of data analysis

TABLE 6. List of steps (check sheet) for validation protocols

	Task	Comments
☐	**1. Identify the process to be validated**	
☐	**2. Determine the appropriate test to conduct**	What is the question to be answered?
☐	**3. Determine if natural microflora or inoculated surrogates will be used**	Use of surrogates may require an external laboratory
☐	**4. Design the in-plant demonstration**	Keep in mind the number of samples and the number of independent replications
☐	**5. Determine the appropriate methods of analysis**	Determine the minimum detection limit, sensitivity and specificity of the analytical method
☐	**6. Schedule the in-plant demonstration**	Consider production schedules and product disposition issues
☐	**7. Begin the in-plant demonstration**	Be sure that the samples are collected in accordance with recognized procedures and transported to the laboratory in a timely fashion
☐	**8. Analyze the samples**	Assure that laboratory quality assurance programs are in place
☐	**9. Overview of the data**	Look for obvious errors in the data
☐	**10. Analyze the data in-plant demonstration**	Use appropriate statistical tools to evaluate the results of the
☐	**11. Draw the appropriate conclusions**	Do not generalize the results beyond the limits of the demonstration (process and facility specific)
☐	**12. Prepare a detailed report in a timely fashion**	The demonstration is not complete until the report is written

CONCLUSION

Validation is a critical aspect of HACCP and should be conducted to assure the safety of the product being produced in a particular food processing operation. Food safety regulations require that processing operations implementing HACCP systems should validate their critical control points and the overall HACCP system. The goal of a food safety management system such as HACCP is to ensure the safety of the food products being produced under that system. Validation includes ensuring that the CCPs within the process are achieving their intended purpose.

These validation activities should be properly designed and executed in the processing operation to evaluate the effectiveness of the CCPs as implemented in preventing, reducing and/or eliminating the food safety hazard and that the products produced under the HACCP plan are safe.

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WEB SITES

http://www.meathaccp.wisc.edu/validation/index.html

http://meatsci.osu.edu/HACCPsupport.html > "Supporting Documentation Materials for HACCP Decisions"

http://www.fsis.usda.gov/science/HACCP_Validation/index.asp and

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